THE USE OF NON-INVASIVE TECHNIQUES TO ESTIMATE BONE MINERAL CONTENT AND BONE STRENGTH IN THE HORSE

BY

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1986

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ACKNOWLEDGMENTS

My sincere appreciation is expressed to Dr. E. A. Ott, chairman of the supervisory committee, for directing my academic program and research and for allowing me to pursue other equine interest to round out my education. Dr. E. L. Johnson and Dr. S. Lieb were also very helpful.

Special thanks are also extended to Dr. C. S. Ammerman, Dr. R. L. Asquith, Dr. S. Lieb, Dr. G. Pietrowski and Dr. P. W. Poulos for their willing assistance in serving on the supervisory committee.

Additional appreciation is extended to Dr. R. L. Asquith, Dr. C. S. Ammerman, J. Kivipelto, C. Roden, Dr. J. W. Harvey and F. K. McNulty for expertise and assistance with the iron-phosphorus study.

The engineering expertise of Dr. G. Pietrowski and Dr. G. Millec helped develop an area of major emphasis for this study. A special thanks is extended to Dr. G. Millec for his efforts with mechanical testing and geometrical analysis.

Special thanks are extended to Dr. P. W. Poulos for providing the metacarpals and for directing me in a study of the literature that led me to expand this project in both depth and scope.

Sincere thanks are expressed to Dr. R. Tessa and Dr. P. Figueroa for technical assistance with photon absorption and ultrasound.

D. E. Campbell and S. N. Williams assisted with computer programs and statistical analysis and P. Dolder typed the dissertation. Their proficiency was a tremendous help.

The assistance of N. Wilkinson and E. Smith with the actual laboratory analysis is greatly appreciated. The interest and support of fellow students and friends S. N. Williams, N. Wilkinson, F. Milas, B. Pitzer, D. R. Campbell, W. J. Anderson, G. D. James, M. A. McGuire, P. Dolder and E. A. Rathgeber helped immeasurably in the completion of this project.

The greatest debt of gratitude is owed to my parents whose support and encouragement even in the most difficult times never wavered once.

TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS	iii
LIST OF TABLES	vii
LIST OF FIGURES	viii
LIST OF ABBREVIATIONS	x
ABSTRACT	xi
CHAPTER	
I REVIEW OF BONE STRUCTURE AND FUNCTION.	1
Introduction	1
Structure of Bone.	2
Bone Growth and Histology.	5
Bone Remodeling.	12
II MECHANICAL PROPERTIES OF BONE	14
Breaking Strength of Equine Metacarpals	26
Materials and Methods.	26
Results and Discussion	29
III THE USE OF NON-INVASIVE TECHNIQUES TO PREDICT BONE MINERAL CONTENT AND STRENGTH IN THE HORSE	43
Radiographic Photometry	43
Photon Absorptiometry.	49
Ultrasound	55
Evaluation of Equine BMC and Bone Strength	58
Experimental Procedure	59
Result and Discussion.	62
Radiographic Photometry.	69
Photon Absorption.	83
Ultrasound	83
IV BONE DEVELOPMENT AND MINERAL INTERACTIONS.	95
Iron Supplementation In Horses	95
Iron Absorption	96
Iron Metabolism	97
Iron Storage	98
Iron Toxicity	98

Iron Interactions 99
Iron-Phosphorus 99
Iron-Copper-Zinc-Manganese. 101
Influence of dietary iron on growth, tissue mineral composition, apparent phosphorus absorption and chemical and mechanical properties of bone in ponies 103
Experimental Procedure 104
Results and Discussion 110
Animal Growth 110
Blood and Serum Composition 112
Tissue Mineral Composition 116
Apparent Absorption of Calcium and Phosphorus 119
Bone Growth and Development 119

REFERENCES. 125

BIOGRAPHICAL SKETCH 134

LIST OF TABLES

Table		Page
1	BONE MINERAL CONTENT AND MECHANICAL PROPERTIES OF BONE BY AGE GROUP	31
2	MOMENT OF INERTIA	55
3	CORRELATION COEFFICIENTS FOR SCABS VS AREA INDICES	65
4	CORRELATION COEFFICIENT FOR BMC VS AREA INDICES	74
5	RADIOGRAPHIC PHOTOMETRY - CORRELATION	79
6	PHOTON ABSORPTION AND ULTRASOUND CORRELATION COEFFICIENTS	82
7	CORRELATION COEFFICIENTS FOR BMC AND BREAKING STRENGSH VS ULTRASOUND	88
8	MEANS FOR ULTRASOUND	94
9	EXPERIMENTAL DESIGN	105
10	COMPOSITION OF BASAL DIET	106
11	ANALYSED COMPOSITION OF EXPERIMENTAL DIETS	108
12	EFFECT OF DIETARY IRON ON INTAKE AND WEIGHT GAIN	111
13	EFFECTS OF DIETARY IRON ON CERTAIN HEMATOLOGICAL VALUES IN PONIES	113
14	EFFECT OF DIETARY IRON ON SERUM COMPOSITION OF PONIES	114
15	EFFECT OF DIETARY IRON ON THE TISSUE MINERAL COMPOSITION OF PONIES	117
16	EFFECT OF DIETARY IRON ON APPARENT ABSORPTION OF CALCIUM AND PHOSPHOROS	120
17	EFFECT OF DIETARY IRON ON PROPERTIES OF THIRD METACARPALS OF PONIES	121
18	EFFECT OF DIETARY IRON ON PHOTOMETRIC DENSITOMETRY ESTIMATES OF BONE MINERAL CONTENT AND CORTICAL AREA OF METACARPALS OF PONIES	124

LIST OF FIGURES

Figure		Page
1	DIAGRAM OF BENDING TEST	17
2	FORCE-DEFORMATION CURVE	19
3	CROSS-SECTION OF METACARPAL	28
4	BMC VS AGE	30
5	BREAKING STRENGTH VS AGE	32
6	BMC VS BREAKING STRENGTH	34
7	MOMENT OF INERTIA (SCADS) VS MOMENT OF INERTIA OF A CIRCLE	36
8	MOMENT OF INERTIA (SCADS) VS MOMENT OF INERTIAL OF AN ELLIPSE	37
9	BMC VS BREAKING LOAD	39
10	FORCE-DEFORMATION CURVES FOR METACARPALS FROM HOUSES OF VARIOUS AGES	40
11	BMC VS AGE	63
12	BMC VS AGE	64
13	SCADS VS RADIOGRAPHIC AREA INDEX	66
14	SCADS VS CIRCULAR AREA INDEX	67
15	SCADS VS ELLIPTICAL AREA INDEX	68
16	BMC VS RADIOGRAPHIC AREA INDEX	70
17	BMC VS CIRCULAR AREA INDEX	71
18	BMC VS ELLIPTICAL AREA INDEX	72
19	BMC VS SCANS	73
20	BMC VS MEDIAL FEAK	75

21	BMC VS LATERAL PEAK	76
22	BMC VS MIDPOINT	77
23	BMC VS RADIOGRAPHIC PHOTOMETRY	78
24	BMC VS PHOTON ABSORPTION	81
25	BMC VS ULTRASOUND	84
26	BREAKING STRENGTH VS ULTRASOUND	85
27	ELASTIC MODULUS VS ULTRASOUND	86
28	BMC VS ULTRASOUND	89
29	BMC VS XBI/ULTRASOUND	90
30	BMC VS CAI/ULTRASOUND	91
31	BMC VS EAI/ULTRASOUND	92
32	BMC VS SCADS/ULTRASOUND	93

LIST OF ABBREVIATIONS

AP	Anteriorposterior
BMC	Bone Mineral Content
BS	Breaking Strength
C	Distance From Neutral Axis to Outermost Particle
Ca	Calcium
CAI	Cirrular Area Index
Cm	Centimeter
CF	Crude Protein
Cu	Copper
d	Day
D	Deformation
E	Modulus of Elasticity
F	Force
Fe	Iron
GPa	Gigapascal
Hb	Hemoglobin
Hct	Hematocrit
I	Moment of Inertia
IC	Moment of Inertia of Circle
IE	Moment of Inertia of Ellipse
in	Inch
K	Potassium
kg	Kilogram
L	Length
LA	Lateral Peak
lb	Pound
LM	Lateromedial
m	Month
Mg	Magnesium
ML	Medial Peak
mm	Millimeter
Mn	Manganese
Mo	Molybdenum
MPa	Megapascal
P	Phosphorus
PTH	Parathyroid Hormone
RBC	Red Blood Cell
SCADS	Computer Program
Se	Selenium
TIBC	Total Iron Binding Capacity
UIBC	Unbound Iron Binding Capacity
wk	Week
XAI	Radiographic Area Index
yr	Year

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
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December, 1985

Chairman: Dr. E. A. Ott
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Photon absorption, radiographic photometry and ultrasound were tested on a common group of equine metacarpals to determine their ability to predict bone mineral content (BMC) and breaking strength. Tests were performed on 25 pairs of metacarpals collected from equine cadavers ranging in age from 1 d to 27 yr.

The relationships between BMC, age, cortical area and the mechanical properties of bone were established. Geometrical properties of cortical area and moment of inertia were calculated using indices and by computer analysis. Breaking strength and BMC were found to increase dramatically during the first year. Maximum breaking strengths were reached in horses between 1.5 to 4 yr of age, while maximum BMC and cortical area was not reached until 5 to 13 yr.

Photon absorption proved to be the most accurate technique for predicting BMC ($r = .991$; $p<.001$). Radiographic photometry was also a

good predictor of BMC ($r = .948$, $p<.001$). Radiographic photometry has the advantage of providing morphometric measurements of cortical area. The effective ultrasound velocity, calculated by dividing the outside bone diameter by ultrasound transmission time correlated well with BMC ($r = .923$, $p<.001$) and breaking strength ($r = .820$, $p<.001$). Improvements in correlations between BMC and ultrasound velocity were accomplished by using cortical areas for the distance traveled by the ultrasound signal.

A study was designed to determine the effects of excess iron supplementation on phosphorus and the trace minerals, copper, zinc and manganese, all known to be essential for proper bone development. Twelve weanling (6) and yearling (6) ponies were fed diets supplemented with 0, 500 and 1000 mg/kg iron as ferric citrate. Iron supplementation did not effect growth, packed cell volume, hemoglobin, red blood cells or serum levels of phosphorus, iron, calcium, manganese or copper. However, serum zinc was lowered ($p<.05$) in the 500 mg/kg group. Liver zinc values were lowered ($p<.05$) by iron supplementation. Supplementation with 100 mg Fe/kg lowered copper and manganese in the spleen. Apparent phosphorus absorption, BMC and breaking strength of third metacarpals as estimated by non-invasive techniques were not adversely affected by iron supplementation.

CHAPTER I
REVIEW OF BONE STRUCTURE AND FUNCTION

Introduction

Modern animal production practices have increased the importance of optimum bone development and strength at an early age along with the need for prolonging the useful life of breeding animals. Every phase of livestock production has realized the need for quality bone development. Confinement operations and the push for maximum production in swine, dairy and poultry have created situations where optimal bone strength is important to the performance of the animal.

Although the animal industry as a whole is interested in achieving optimum bone strength, no group is more concerned than horse producers. In present production systems young horses are placed in rigorous training before their skeleton has matured. Training usually begins as long yearlings, these horses may be raced or enter the highly competitive futurities by two years of age. Either training regime places tremendous stress on immature bone, joints and tendons. Track veterinarians report that the chance of injury same time during a horse's racing career is nearly 100% (Jones, 1984).

Members of the horse industry would like to be able to evaluate skeletal soundness and predict the potential for lameness problems. Trainers are interested in monitoring bone development in response to training. Their main goal is to avoid the almost inevitable breakdown of young horses. Research in the area of nutrition and bone development

in horses is continuing as demands upon the animal change. Exercise physiology and bone development have become an expanding area of equine study.

Horse producers, veterinarians and researchers have focused on developing a non-invasive technique that can be used to predict the strength of bone. The present project compares the effectiveness of photon absorption, X-ray radio densitometry and ultrasound techniques for predicting BMC and bone breaking strength. Twenty-five pairs of equine third metacarpals (McIII) from horses of varied breeds and ranging in age from 1 d to 27 yr of age were used.

A second study is included that makes use of the three non-invasive techniques for determining bone mineralization and strength in weanling and yearling ponies. The project involved a nutritional study of the effects of excessive iron supplementation on phosphorus absorption and bone development. A phosphorus balance study was conducted during a 90 d feeding trial. The ponies were weighed and blood samples drawn at 28 d intervals. Blood samples were analysed for hemoglobin (Hb), hematocrit (Hct), red blood cell (RBC), white blood cell (WBC), total iron binding capacity (TIBC), unbound iron binding capacity (UBIC), and certain serum minerals. Metacarpals, liver and kidney tissues were collected at the end of the trial for mineral analysis and bone breaking strength tests.

Structure of Bone

The outward appearance of bone gives the impression of a static, lifeless, stone like material with the sole function of support. However, the appearance of bone is deceiving. While support is the major function of bone, bone is a dynamic physiological system.

Bone houses the marrow responsible for production of RBC. Bone is also responsible for calcium (Ca) and phosphorus (P) homeostasis. Immediate control of Ca levels in the blood is accomplished by parathyroid hormone (PTH) effects on Ca and P absorption in the proximal tubules of the kidney. Vitamin D and PTH mediated absorption of Ca and P in the intestine are also important to maintenance of proper Ca and P status.

Every gram of bone has an exposed surface of several square meters providing it with a tremendous surface area (Wilson, 1972). The large surface area is important to Ca metabolism. By storage, resorption and mobilization of Ca under control of PTH and calcitonin bone is responsible for the long term homeostasis of this mineral.

Some insight into the function of this seemly inert material can be obtained by examining the structure of bone. The bone cells are responsible for both the structural and metabolic functions of bone tissue. Within mineralized bone lie the osteocytes (mature bone cells) with a system of blood vessels. The blood vessels supply nutrients and provide a system of communication delivering hormones and mesenchymal cells, capable of differentiating into osteoprogenitor cells.

The turnover of bone is important to the performance of its structural and metabolic functions. By examining some of the factors which effect remodeling of bone we can begin to understand the dynamics of bone. Mature bone is also constantly being quenched and reformed. This internal remodeling is a normal process and may occur without changing the gross anatomic structure of the bone. Internal remodeling may occur in response to any of the following stimuli: altered vascular

relationships that require the repositioning of vessels within compact bone to insure adequate nutrition for osteocytes, response to altered biomechanical stress, the mobilization of bone mineral in response to dietary deficiencies, immobilization and/or disease, and altered endocrine function (Banks, 1981). Structural remodeling is the removal of immature bone tissue of very young bones and its replacement with compact tissue of a mature bone. This process will be discussed in more detail when bone growth and histology is covered.

Trabecular bone is more labile than compact bone. Trabecular remodeling activity may be as much as three times higher than compact bone. The entire remodeling process, while demonstrating the dynamics of bone, is not a rapid process. The time required from resorption to reformation of a mature Haversian system may require months (Banks, 1981).

In order for normal skeletal volume to be maintained, there must be a balance of activity between bone formative and resorption processes. The homeostatic control of remodeling is achieved by the balanced activity of osteoblasts and osteoclasts.

Osteoblasts are osteogenic cells that line the periosteal and endosteal bone surfaces. The primary function of the osteoblast is synthesis and secretion of organic matrix of bone and the initiation of matrix mineralization. The organic matrix consists of collagen and ground substance. Ground substance is a protein, polysaccharide and lipid complex thought be involved with the initiation of mineralization.

Osteoclasts are specialized multinucleated cells responsible for bone resorption. They can be found on the surfaces of bone in cavities

called Howships lacunae. An adaptive modification in the plasma membrane, the brush border, is thought to be capable of removal of both minerals as well as the digestion of the organic matrix (Benhn, 1981). The brush border is a cellular border adjacent to the bone with numerous finger like projections. An ultra structure view of the bone surface beneath the brush border appears irregular. On either side of the brush border there is a smooth, clear transitional zone. This zone may function as a seal between the cell and the bone surface to confine the organic acids and lysosomal enzymes responsible for bone resorption to the area of the brush border.

Osteocytes are osteoblasts that have become embedded in bone. Microscopically osteocytes are similar to osteoblasts. They continue the same osteoblastic activity of maintaining the matrix only to a lesser degree (Hsu and Cormack, 1979). Osteocytes may also possibly function in the resorption of bone.

Bone Growth and Histology

A microscopic examination of compact bone reveals a lamellar structure with many vascular channels (osteons or haversian systems), pores (lacunae) and resorption spaces. The basic unit of bone is the osteon, a tubular structure about 250 microns in diameter. The center of the osteon contains one or more small blood vessels. Surrounding the vascular canal several layers of concentric lamellae form the walls of the Haversian systems. Osteocytes lie in lacunae between the lamella and are connected to other osteocytes and blood vessels by canals (canaliculi). Each osteon is enclosed by a distinct cement line (Wilman, 1972). The osteon is common to all bone, differences between

bones are due to the number of osteocytes and lamellae comprising the osteons.

In order to better understand changes taking place in bone due to age, mechanical forces, nutrition, hormone response and diseases or injury some discussion of the histology of bone development is helpful.

During embryologic development osteogenesis (ossification) begins in two sites, 1) areas of ordinary mesenchyma (intramembraneous ossification), and 2) within cartilage models of bone (endochondral ossification) (Ham and Cormack, 1979). Regardless of the area in which bone develops there are no significant differences in the process or the resulting kind of bone.

The bones of the skull, mandible and clavicle are formed by intramembraneous ossification. Before ossification begins the mesenchyma consists of widely separated more or less triangular or star-shaped cells with processes that often connect with those of adjacent mesenchymal cells. In one or two sites in the mesenchyma where blood capillaries are present the center of osteogenesis develops. The mesenchymal cells become more rounded and their processes connect with the processes of other developing cells. These cells later differentiate into osteoblasts and surround themselves with matrix. The cytoplasmic processes act as molds around which the organic matrix is poured. Matrix is then calcified by absorbing Ca salts. Canaliculi and lacuna are filled with fluid from the capillaries and the osteoblast then differentiate into osteocytes.

Further growth of bone occurs by trabeculae extending in a radial fashion from the center of osteogenesis, the trabeculae grow and join

each other in an overlapping network pattern called cancellous bone. Cancellous bone may develop further into compact bone. Compact bone is mature bone in which more space is occupied by bone than soft tissue and blood vessels. This development is accomplished by the osteogenic cells that line all surfaces of the anastomosing trabeculae. The osteogenic cells multiply and the innermost layer differentiates into osteoblasts. The osteoblasts then secrete the organic matrix which is calcified and forms a new layer of trabecular bone. The trabecula become thicker and the spaces smaller. The layered structure is built up around a space containing one or two vessels. These concentric structures are the Haversian systems.

Ham and Cormack (1979) emphasize that bone formed as a result of intramembraneous ossification is no different from that formed by endochondral ossification. However, there are two types of bone distinguished by the arrangement and relative amounts of intercellular substance. Immature bone has more intercellular substance and fewer osteocytes and no growth centimes it is replaced by mature bone with increased number of osteocytes and mineral.

Endochondral ossification occurs in what will become the weight bearing bones in the mature animal. As the mammalian embryo develops mesodermal outgrowths covered with ectoderm appear as limb buds. The mesenchymal cells increase in number and pack together to form an outline of the future bone. In the case of the parked mesenchyme, cells begin to differentiate into chondrocytes. The chondrocytes secrete the cartilage matrix. While the mesenchymal cells of the model differentiate into chondrocytes, mesenchymal cells around the periphery condense to form the perichondrium.

The cartilage model grows as the embryo gets larger by interstitial and appositional growth. Appositional growth originating from the perichondrium lengthens and thickens the model. Interstitial growth is responsible for lengthening the model near the ends by cell division of chondrocytes. Extensive growth is occurring at the ends of the model, chondrocytes in the midsection have time to mature. This area then becomes calcified.

At the same time that the events described above are taking place in the midsection of a cartilage model, the progressive development of the vascular system of the embryo leads to the perichondrium of the model being invaded by blood vessels. Increased oxygen supplied by the capillaries will rouse cells that until now have differentiated into chondrocytes to differentiate into osteoblasts and osteocytes. The perichondrium now becomes the periosteum and a thin layer of bone is laid down around the shaft of the model.

After the calcification of the cartilage in the midsection of the model, cavities develop within this area by dissolution of the cartilage matrix. Blood vessels from the periosteum invade the mineralized cartilaginous matrix. A single, predominant vessel is responsible for the vascularization of this previously avascular cartilaginous model. This nutrient artery and its associated mesenchymal cells differentiate into osteoprogenitor cells to become the periosteal bud (Banks, 1981)

When the osteogenic cells and blood vessels of the periosteal bud reach the midsection of the cartilage model, they form the center of ossification. Capillary loops from the periosteal bud with bone cells, advance into the calcified matrix removing cartilage by osteoclasis and

creating new surfaces for bone formation. The first bone formed in this area is cancellous. As the subperiosteal bone becomes thicker osteoclasts reach the cancellous bone and components of the periosteal bud develop the marrow.

At this point the midsection is cancellous bone covered by a shell of subperiosteal bone with increasing amounts of developing marrow tissue. This midsection is called the diaphysis. The epiphysis at the end of the bone remains cartilaginous at this time. Most of the long bones develop from at least three ossification centers. The primary ossification center is responsible for the diaphysis. A center of ossification may also develop in each of its cartilaginous epiphyses. Chondrocytes mature in the center of the ends of the model and the intercellular substance then becomes calcified, causing the chondrocytes to die. Dissolution of matrix and formation of cavities is followed by invasion of tissues consisting of capillaries and osteogenic cells. The osteogenic cells form osteoblasts that deposit bone on the remnants of the calcified cartilage matrix. The process of ossification then spreads out from the epiphyseal center in all directions. Ossification stops short of replacing all the epiphyseal cartilage. Cartilage remains as an articular surface of joints and cartilage remains as a disk or plate between the bone forming in the epiphyseal center and the bone forming in the diaphyseal center. This transverse disk of cartilage separating the epiphyseal bone from the diaphyseal bone is termed the epiphyseal disk, plate or physis.

Interstitial growth of cartilage cells in the epiphyseal plate is responsible for longitudinal growth of long bones. The growth of

cartilage cells of the epiphyseal plate thickens the plate and displaces the bone of the epiphysis away from that of the diaphysis. The result is that bone length is increased. Only when long bone growth ceases does interstitial growth of the cartilage of the epiphyseal plate stop and the plate is replaced by bone.

The histological organization of the epiphyseal plate is similar to the ossification center of the cartilage model. Four zones of cartilage cells are identifiable in the epiphyseal plate.

The zone of resting chondrocytes lies immediately adjacent to the bone of the epiphysis. The cartilage in this zone does not divide or participate in the growth of the epiphyseal disk. Its primary function is to attach the plate to the bone of the epiphysis. A second function may be that by diffusion of nutrients through the plate it provides a nutrient source for the other zones.

The second zone is composed of proliferating chondrocytes. These cartilage cells appear as stacks of thin, wedge-shaped cells that are actively mitotic. They are responsible for longitudinal growth and adding new cells to replace those lost to metaphyseal osseous replacement. The cells are stacked in columns perpendicular to the plate.

The third zone contains maturing chondrocytes. These cartilage cells are also arranged in columns becoming more mature as they progress from the zone of proliferating cartilage to the diaphysis. As the cells mature they become large and expand the epiphyseal disk longitudinally. The epiphyseal plate is therefore expanded both by proliferation of cells in the second zone and by maturation of cells in the third zone.

The chondrocytes of this zone produce alkaline phosphatase and calcium containing matrix vesicles. As this zone merges into the next the intercellular substance around the cartilage cells becomes increasingly calcified.

The zone of calcified cartilage is the fourth zone. This zone is adjacent to the diaphysis. Capillaries and osteogenic cells from the diaphysis invade the columns of chondrocytes when the chondrocytes in the newly calcified zone die and the partitions between their empty lacunae disintegrate. The invading osteogenic cells multiply and differentiate into osteoblasts. Osteoblasts arrange themselves along the remnants of the cartilaginous trabeculae and cover them with a layer of bone.

The epiphyseal ends of the trabeculae formed by the growth plate are constantly elongated, yet at the periphery of the bone this zone of trabeculae stays about the same length. This means that as rapidly as bone is added at the epiphyseal end it must be removed from the diphyseal end. This is accomplished by osteoclasts wrapped around the free ends of trabeculae. The metaphyseal regions at the area of attachment to the epiphysis has a greater diameter than the narrower waist of the bone shaft. As the bone grows in length the diameter of the metaphyseal region must be decreased. This requires that bone be resorbed from the outside of the flared portion by osteoclast and built up on the inside by osteoblasts.

In the zone of calcifing cartilage the chondrocytes are arranged in longitudinal rows separated from one another by intercellular substance. The longitudinal rows of cartilage cells are the forerunners

of the haversian systems of compact bone. The development of mature osteone and the fate of the cartilaginous interlamellar spaces are factors in the development of bone that lead to increased mineralization and strength as the animal ages.

As the cartilage cells in the rows of longitudinal tunnels mature and die the tunnels fuse into one large tunnel. Osteogenic cells, osteoblasts and capillaries invade these tunnels and bone is deposited on their surfaces. Appositional growth results in successive layers of bone being deposited inside the tunnels. Several concentric layers of lamellar bone are laid down around a central blood vessel.

Since Haversian systems of developing bone are formed in cartilaginous tunnels the space between tunnels is cartilaginous also. As the animal gets older the space between adjacent Haversian systems is filled with bone.

There is no cartilage between Haversian systems in older bone. As long bone grows in width new layers are added at the periostium by appositional growth, while bone is being resorbed from the marrow cavity. The result is that the bone as a whole is widened as the marrow cavity diameter is increased.

Bone Remodeling

The growth of long bone is the bone is designed to produce a bone of maximum strength while preserving the advantage of light weight. Growth in length and width is a form of remodeling. The bone of a young animal is not the same as that found in a mature horse. During the growth process structural remodeling causes a complete turnover and replacement of bone tissue.

Structural remodeling also occurs due to mechanical forces. Wolff's Law states that remodeling takes place so that skeletal form and mass adjusts to provide optimum bone strength (Lauyon, 1982).

Frost (1973) building on Wolff's Law of structural remodeling developed the Flexure Drift Law. He stated that during bone deposition, lamella orient parallel to the direction or resultant of the greatest tension and/or compression stress. Frost (1973) proposed that this force response characteristic accounts for the anatomic conformation of various bones.

CHAPTER 11
MECHANICAL PROPERTIES OF BONE

Within minutes after the birth of a foal he tests the strength of his limbs. Throughout his life his bones will be subjected to a variety of forces. The response of bone to these forces is a function of the mechanical properties of bone (Evans, 1973). Bone structure both responds to and is derived from its mechanical properties (Frankel and Burstein, 1970).

Animal nutritionists have used the mechanical properties of bone, such as the breaking strength, for determining the bioavailability of minerals and establishing nutrient requirements (Haugh et al., 1971, Schryver, 1978, Crenshaw et al., 1981a). More recently researchers have become interested in the mechanical properties of bone as they relate to age, growth and development, remodeling, exercise and training, gait analysis and interaction with track surfaces. Before discussing research in these areas it may be appropriate to review some of the basic principles of mechanics as they relate to the properties of bone.

One of the basic terms used in mechanics is force. A force is simply a push or a pull. There are four characteristics that describe a force: 1) magnitude, 2) line of application, 3) sense, (e.g. upward or downward along a vertical), and 4) the point of application (Frankel and Burstein, 1970). The magnitude of a force can be measured in kilograms, pounds or newtons. There are three types of force: tension, compression and shear.

When a force is applied to a body it creates a stress and strain. Strain is the change in the linear dimensions of a body as the result of the application of a force. Stress is a measure of the loading intensity, expressed as force per unit area of bone

Bone can be tested as a structure or as a material. When bone is tested as a structure a force is applied until failure. The breaking load is the force at which any further increase in force results in failure of than bone. Breaking load is a structural test of the bone. Stress is a material property of bone or the osseous material of bone. Stress results from an applied load. Breaking strength is the maximum stress the bone material can withstand.

It is important to note that although often used interchangably in the literature, breaking load and breaking strength are different. Breaking load is the force required to break a bone. Breaking load is determined by both the size and quality of the material. To compare bones of different size, geometrical effects must be included and bone can be tested as a material. In order to calculate breaking strength the moment of inertia (I) must be determined. This includes the transverse area of the bone and the shape of the area over which the force is applied (Grashaw et al. 1981b).

Because of the irregular shape of bone there are difficulties in determining the area and geometry for calculation of the moment of inertia. Researchers deal with these problems by machining small uniform sections of bone and by using equations to estimate area and geometrical properties of bone. The mechanical properties of osseous material can be studied by machining small uniform sections for

testing. Schryver (1978) used this technique in a study of Ca and P relationships and the effect of exercise on ponies. Machined sections overcome the problems of determining the geometry of bone. When machined sections of well defined geometry are used, bone is being tested as a material. If the whole bone is tested its structural, i.e material and geometrical, properties are tested. This test is more representative of forces acting on bone in the animal (Evans, 1973).

The most common test used to evaluate the mechanical properties of a long bone is a flexure test. In a flexure test the bone is rested on two supports and a force is applied from above. Breaking strength (BS) of the bony material is calculated from a bending test as follows:

$$BS = \frac{P\ L\ C}{4\ I}$$

In the bending test (figure 1) BS (kg/cm^2 or MPa) can be related to force (P) (kg) by the formula given above. Length (L) (cm) is the distance between the supports. When a bone is bent as shown in figure 1, the material in the upper half of the bone will be compressed, while the material in the lower half will be in tension. The neutral axis is the plane of material in the center which is neither compressed nor stretched and C (cm) is simply the distance from the neutral axis to the outermost particle, at the section where the force is applied. The moment of inertia (I) (cm^4) is a property of the cross sectional area and geometry of bone. The further from the center the material is distributed, the greater the I. The moment of inertia can be estimated using equations of known geometrical shapes or by computer analysis.

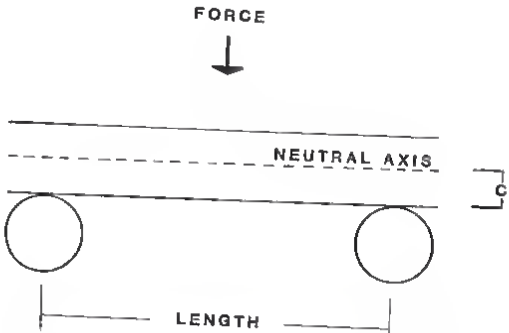

FIGURE I. DIAGRAM ILLUSTRATING EQUINE THIRD METACARPAL WITH FORCE APPLIED TO THE POSTERIOR SURFACE. NEUTRAL AXIS IS REPRESENTED BY DASHED LINE IN CENTER OF BONE. C IS THE DISTANCE FROM THE NEUTRAL AXIS TO THE EXTREME OUTER PARTICLE. LENGTH IS THE DISTANCE BETWEEN ROLLER SUPPORTS.

To overcome problems of determining bone geometry a computer program has been designed to calculate the actual area and I of cross-sections of bone (Piotrowski and Kellman, 1973). Cross-sections are digitized using a microcomputer with a graphics tablet, this data is then transferred to a main frame computer program (SCADS) which computes the area moment of inertia without assuming any geometrical shape.

In a study of the relationship between age and breaking strength Bynum et al. (1971) tested whole third metacarpals in bending. He used the equations for an ellipse to calculate the moment of inertia. In a second study of the age-breaking strength relationship El Sherafa et al. (1979) used the equation for a circle to calculate the moment of inertia. Breaking strength values from Bynum et al. (1971), Schryver (1978) and El Sherafa et al. (1979) were comparable with some of the differences possibly attributed to approximations made in estimating the moment of inertia.

When bones are tested in a flexure test, force is plotted against deformation produce a characteristic force-deformation curve (figure 2). In the initial linear part of the curve elastic deformation occurs. If the force is removed from the bone during this portion of the curve the bone will return to its original shape. Beyond the proportional limit, or linear portion of curve, is the area of plastic deformation where permanent deformation (microdamage) occurs. Breaking strength is the stress at which the bone breaks.

The modulus of elasticity (E) is the ratio of stress to strain, and is measured in kg/cm^2 or MPa. The modulus of elasticity is a measure of the stiffness of bone in the linear elastic region. It is determined from a bending test (figure 1) by the following equation:

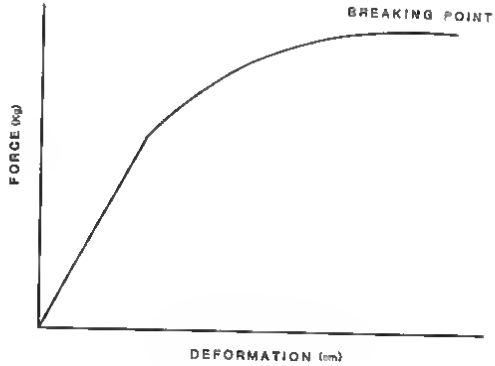

FIGURE 2 FORCE-DEFORMATION CURVE

$$E = \frac{F\,L}{4B\,I\,D}$$

Deformation (D) is obtained from the force-deformation curve, F, L and I are obtained as previously discussed.

When testing the mechanical properties of bone it is important to compact deformation rates. McElhaney (1966) found that as the loading rate was increased the capacity for long bones to absorb energy was increased. Thus, as loading rates increased breaking strengths also increased. Bone may demonstrate nearly twice the stiffness at high speed loading rates compared to slow rates (Frost, 1973). Evans (1973) points out the significance of loading rate as a safety factor in reducing the possibility of fracture. Bynum et al. (1978) failed to report the rate of deformation.

Recently there have been many studies to determine the physical forces to which equine bones are subjected, but relatively few studies of the mechanical properties of bone and the loads they can withstand. In order to assess the many factors which may influence the mechanical properties of bone strength a data set of normal standards must first be obtained. The process of bone development dictates that these standards must take age into consideration.

Bynum et al. (1971) performed flexure tests on 37 whole equine metacarpals from horses ranging in age from 3 months to 30 years. The bones were tested by 3 point bending on a testing machine (Instron Corporation) until failure. The load was applied at the midpoint of the metacarpal. Cross-sectional geometry was determined to be closely approximated by 2 ellipses. For the calculation of the moment of

inertia the equation for an elliptical sections was modified to account for offsetting of the medullary cavity.

Bynum et al. (1971) reported breaking strengths increased from 140 MPa (20 ksi) at three months of age to 280 MPa (40 ksi) at six years of age. As the animal aged from six to 30 years the breaking strength decreased to a level just above 140 MPa. The modulus of elasticity followed a similar pattern. The average modulus of elasticity was 12 GPa (1.7×10^6 psi). The authors reported that both stiffness and fragility of the equine metacarpals increased with increasing age.

A second study of the relationships and among BS, age, sex torsion and cortical area in the equine metacarpal (El Shafie et al., 1979). El Shafie et al. (1979) tested equine metacarpals from 41 horses ranging in age from one day to 33 years by three point flexure test. The load was applied at approximately the midpoint of the bone at a deformation rate of 2.5 cm/min.

El Shafie et al. (1979) assumed the geometry of McIII to be a tube and used the equation for a circle to calculate the moment of inertia (I) measured in cm^4:

$$I\ (cm^4) = \pi\ (D^4 - d^4)/64$$

where D = total diameter and d = marrow diameter.

El Shafie et al. (1979) reported breaking strengths increased from 163 MPa (1660 kg/cm^2) at eight months to 204 MPa (2084 kg/cm^2) at one year. From one year to seven years there was a gradual increase to 229 MPa (2341 kg/cm^2). After seven years of age breaking strengths began to decline until it reached 180 MPa (1980 kg/cm^2) at 33 years of age. El Shafie et al. (1979) also examined the relationship between

ash and breaking strength in the equine. A linear regression analysis of breaking strength and ash showed a positive correlation ($r^2 = .58$).

Vace and Kubela (1959) working with rabbit metatarsals reported that breaking strength increase rapidly with small increases in ash. They fit an exponential curve to their data. Curry (1969) explained the failure of bone to completely mineralize and thus produce a linear relationship between ash and breaking strength by suggesting that mineralization beyond an optimum value would decrease resilience of bone and reduce its ability to resist dynamic loading. Curry (1969) further suggested that the relationship of bone mineral and collagen is responsible for the correlation of breaking strength and ash. He stated that mineral is added to stiffen the bone but at low values the mineral crystals disrupt the collagen molecules when a load is applied. As ash values reach 63 - 68% the organization of the mineral-collagen matrix produces the optimum strength.

The studies of Symms et al. (1971) and El Shorafa et al. (1978) are the only studies where the effect of age and bone strength in horses in bending have been reported. Other studies have used bending tests to determine bone strength in response to dietary treatment and exercise.

Hough et al. (1971) used breaking strength from flexure test to compare the effects of diet and an anabolic agent on 38 yearling ponies. The ponies were allotted to four groups, two groups received either a corn-soybean and oat diet or just oats. The other two treatments were the same diets for groups one and two, with a vit-trace mineral premix added. One half of each group was randomly selected and given an anabolic agent. The ponies remained on trial for one year. At the conclusion of the experiment the ponies were destroyed and the third

metacarpals and third metatarsals were removed. The bones were tested by bending on a testing machine. The load was applied at a rate of 2.54 cm/min (1.0 in/min) to the midpoint of the bone. Hough et al. (1971) reported that there was no difference between the metacarpals and metatarsals with values of 241 MPa (35,000 lb/in^2) and 244 MPa (35,300 lb/in^2) for bending strength, respectively. Though the results were not significant there was a 14.3% reduction in bending strength for metacarpals and an 11.0% reduction for metatarsals for animals receiving the anabolic agent. Females seemed to be more adversely affected than males.

Schryver (1978) conducted a study to determine the bending properties of vertical bone from different anatomic sites and to investigate the effects of diet and exercise on bone strength in ponies. Bone samples were obtained from 12 ponies, 18 months of age. The ponies had been fed one of the three following diets: basal (0.7% Ca, 0.6% P), high calcium and high phosphorus (2.7% Ca, 1.1% P) or high phosphorus (0.7% Ca, 1.4% P). Two ponies from each group were exercised by trotting 16 km a day for four months. Machined sections of bone from the mid diaphysis of the left femur, radius and metacarpus were tested. Schryver (1978) reported that there was little difference in the bending strength or modulus of elasticity from corresponding quadrants of different bones. He found greater differences between the quadrants of each bone than there were between bones. Regression analysis indicated differences in bending strength were positively related to specific gravity. The bones of exercised ponies, males or ponies fed diets containing 0.7% Ca, 0.6% P or 2.7% Ca, 1.1% P tended to be stiffer

and have greater breaking strengths than unexercised ponies, females, or ponies fed diets containing 0.7% Ca, 1.4% P

Evidence that exercise has a positive effect on bone strength in the horse increases the significance of work in the area of bone strain and the forces involved in equine locomotion. Turner et al. (1975) bonded strain gages in vivo to the cranial, caudal, medial and lateral aspects of the equine radius and tibia and to the dorsal, palmar or plantar medial and lateral aspects of the metacarpus and metatarsus in the mid-diaphyseal region. A different horse was used for each bone investigated. Strain changes were recorded while standing, walking and trotting or pacing. Turner et al. (1975) found strains to be highest on the dorsomedial and dorsolateral aspects of the metacarpus and metatarsus.

Calculated forces were highest when trotting or pacing and were 2 to 2.5 times the body weight. Strains were highest for the radius and tibia at the cranial and craniolateral aspects. The radius was subjected to forces 2.5 times the body weight during pacing. The tibia was loaded approximately equally regardless of the type of locomotion studied.

Bertel et al. (1978) developed a model to determine the internal forces in the forelimb of the horse. Input data for the model are external forces between the hoof and the ground, configuration of the digit during the support phase of the gait and internal geometry of the digit. External forces were obtained by a force platform, kinematics by high-speed cinefilms and digit geometry from radiographs. Bertel et al. (1978) predicted that compressive loads at a walk and trot are 13% and

[illegible]5% of maximum compressive strength of the metacarpal which is 71.2 kN (7,256 kg)

Schryver et al. (1978) found the peak axial compressive load in the metacarpal bone for a 450 kg horse to be about 2.4 times body weight for the walk and about 4.9 times body weight for the trot.

Pratscrick and Henderson (1970) designed a forceplate that was attached to the horses hoof to measure forces as a function of time while the horse performed various gaits. Rataloff et al. (1985) designed a similar force plate using a portable integrator and data recorder and a lightar shoe which would not interfere with the normal stride. They looked at vertical forces during the walk, trot and canter on shavings, a sand track and packed clay and found forces on the packed clay to be two to three times the body weight at a canter. Forces recorded on clay were much higher than forces produced on the shavings and a sand track.

With evidence of increased forces at higher velocities and the potential to alter forces by changing the characteristics of the surface, practical application of this information can be made. Chaney et al. (1973) correlated lameness problems with race track surfaces. Impact testing was used to determine the hardness characteristics of five tracks and results were correlated with records of horses reported lame at each track. The authors concluded that the moisture content and dry density of the soil system are important to the response to dynamic forces. Chaney et al. (1973) suggested that a loose top cushion of 5 cm or more could reduce dynamic response values 40 to 60%. They suggest that tracks should have a plant, soft base and a loose cushion.

Breaking Strength of Equine Metacarpals

In order to utilize information on forces created by equine locomotion and their relationship to lameness and track surface, a data base of the effects of age, bone mineral content, geometry and bone strength must be obtained. To increase the data base for equine bone strength and development a study was conducted. The right and left McIII of equine cadavers of varying breeds and ranging in age from one d to 27 yr were tested by bending. The moment of inertia was approximated using the geometry of a circle and ellipse. The actual moment of inertia was also determined by computer program. Bone mineral content was determined for a 2 cm section at the point of break for correlations with breaking strength.

Materials and Methods

Equine third metacarpals were obtained from the University of Florida College of Veterinary Medicine. Accurate records of age, breed and cause of death were available. Only horses the died of acute causes were used to avoid any effect of a chronic debilitating condition. The bones were cleaned of all soft tissue and periosteum, wrapped in saline soaked gauze and frozen at -15°C until testing.

Metacarpals were tested on two supports 13 cm apart. The wet bones were tested by three point bending on a testing machine[1] at a deformation rate of 50 mm/min. The diaphysis of the equine metacarpal demonstrates similar mechanical behavior between 20% and 70% of its length (Piotrowski et al., 1983). The broken bones were reassembled and

[1] Instron Corporation. Model 1125. Canton, MA 02021.

two 2 cm sections were removed one at the point of the break and one 1 cm below the nutrient foramen. These cross-sections were photographed then digitized using a microcomputer with a graphics tablet. The SCADS computer program was used to compute the vertical area and moment of inertia for calculation of breaking strength (Piotrowski and Kellman, 1973). The breaking strength of the bone material was then calculated as described above.

Caliper cross-sectional measurements from bones were anterior posterior (AP) bone diameter (B), AP medullary diameter (b), lateromedial (LM) bone diameter (D), and LM medullary diameter (d) (Figure 3). Equations for the moment of inertia of a circle (MIC) were

$$IC = \pi/64(B^4 - b^4)$$

and moment of inertia of an ellipse (MIE)

$$IE = \pi/64(BD^3 - bd^3).$$

Equations for calculating breaking strength (BS) and elastic modulus (E) are as follows:

$$BS = \frac{(F)\ (L)\ (C)}{4(I)} \qquad E = \frac{(F)\ (L)}{48\ (I)\ (D)}$$

where F = load (kg), L = distance between supports bone rest on (cm), C = distance from neutral axis to extreme outer fiber (cm), I = area moment of inertia $(cm)^4$ and δ = deformation (cm). Force and deformation values were determined from load deformation curves (Figure 2).

Preparation of 2 cm cross-sections involved resting metacarpals in containers made to conform to the size of the bone. Liquid urethane[1]

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[1]Sred foam, Sredsco Auto Marine Supply, Inc., Pinellis Park, FL.

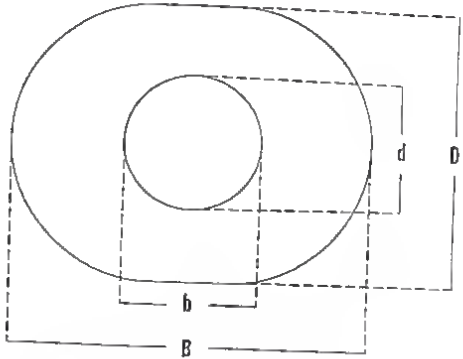

FIGURE 3 CROSS-SECTION OF EQUINE METACARPAL.

which solidifies, was poured around the metacarpal to provide a uniform, flat surface so 2 cm cross-sections could be accurately cut from bone. Cross-sections not conforming to 2 cm length as determined by vernier calipers were ground until proper specifications were attained. The bone cross-sections were ether extracted then ashed at 600 C for 12 hours. Bone ash was expressed as grams per 2 cm section and defined as bone mineral content (BMC).

Results and Discussion

BMC increased with age from 1 d through 4 yr (figure 4). No differences in BMC were found between right and left metacarpals or due to sex. Most of the mineralization occurred during the first yr, BMC were 3 to 4 g for foals and increased to 14 to 16 g for yearlings. There was a more gradual increase in BMC as the animals reached 3 to 4 yr of age (18 to 20 g). During the period of 13 to 27 yr of age there was a tendency for BMC to decline (table 1).

Breaking strength and BMC showed similar relationships with age (figure 5). Breaking strength trends tended to change more dramatically with age, with a rapid increase in strength the first 1.5 yr. As the animal aged past its fourth year there was a decrease in breaking strength. Maximum breaking strengths were reached between 1.5 and 4 yr of age (2151 kg/cm^2, 211 MPa). Bynum (1971) reported maximum breaking strengths occured at 6 yr of age. El Sherafa et al. (1971) reported maximum strengths occurred at 4 to 7 yr of age. It is possible that increased bone strength at younger ages reported in this study may reflect advances in nutrition and the changing management of young horses over the last 15 yr.

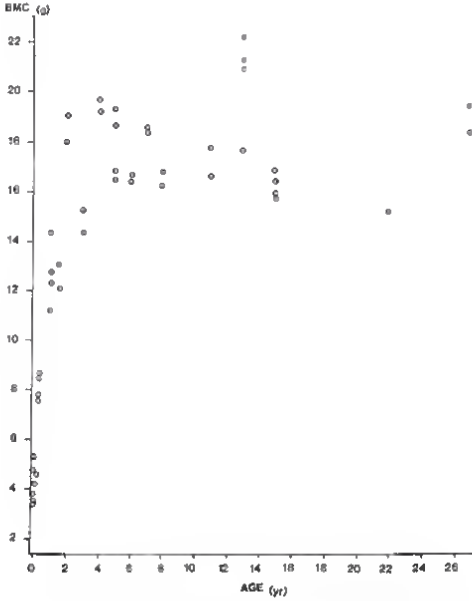

FIGURE 4. RELATIONSHIP BETWEEN BONE MINERAL CONTENT (BMC) (g/2 cm CROSS-SECTION) FROM THE BREAK POINT OF EQUINE METACARPALS AND AGE OF THE HORSE

TABLE 1. BONE MINERAL CONTENT AND MECHANICAL PROPERTIES OF BONE BY AGE GROUP

	1 Day to 1.5 Years[c]	1.5 Years to 4 Years[d]	5 Years to 13 Years[e]	14 Years to 27 Years[f]
BMC g[a]	7.5 ± 3.4	17.6 ± 2.2	18.1 ± 1.9	16.6 ± 1.5
Breaking Load kg	696.0 ± 518.0	2029.0 ± 267.0	1960.0 ± 340.0	1739.0 ± 93.0
Breaking Strength kg/cm^2	1487.0 ± 385.0	2151.0 ± 199.0	2011.0 ± 398.0	1777.0 ± 218.0
Elastic Modulus kg/cm^2	17494.0 ± 6501.0	27152.0 ± 33034.0	30496.0 ± 6457.0	31960.0 ± 3705.0

[a] g/2cm cross section

[b] Mean ± SE

[c] n = 4 males and 4 females.

[d] n = 4 males and 1 female.

[e] n = 3 males and 4 females.

[f] n = 2 males and 3 females

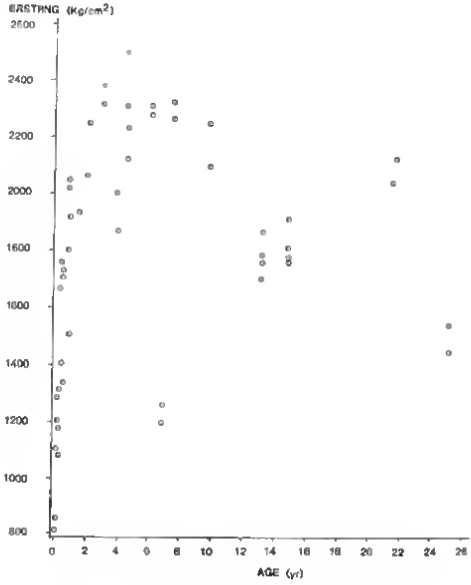

FIGURE 5 RELATIONSHIP BETWEEN BREAKING STRENGTH AND AGE OF HORSE.

As BMC increased, breaking strength increased in an exponential fashion (figure 6) similar to the response reported by Vose and Kubula (1959). The correlation between breaking strength and BMC was .643 ($p<.001$), (table 2). Correlations were not dramatically improved by comparing breaking strength and bone mineral expressed on a volumetric basis (g/cm^3) ($r^2 = .699$, $p<.001$). Currey (1969) explained the failure of bone to completely mineralize and thus produce a linear relationship between BMC and breaking strength by suggesting that mineralization beyond an optimum value would decrease the resilience of bone and reduce its ability to resist dynamic loading.

Comparison of the actual moment of inertia (I) computed by SCABS with estimates using a circle and an ellipse (table 2) indicates that there were no significant differences in IE and I, but IC was different from IE and I. The IC over estimates I by approximately 100%. Regression analysis for I versus IC ($r^2 = .88$, $p<.001$) (figure 7) and IE ($r^2 = .95$, $p<.001$) (figure 8) indicated a close fit of the data for IE and the actual moment of inertia. These findings suggest the area moment of inertia for equine metacarpals can be accurately estimated using the equation for the geometry of an ellipse.

To determine the material and structural properties of bone the whole bone must be mechanically tested (Evans, 1973). Byrum et al. (1971) used an elliptical index for determining the area moment of inertia for breaking strength calculations. El Sherefa et al. (1979) used a circular index to calculate the area moment of inertia of equine McIII bones by flexure test. Schryver (1978) machined small uniform sections of bone for mechanical testing. This type of test is best for

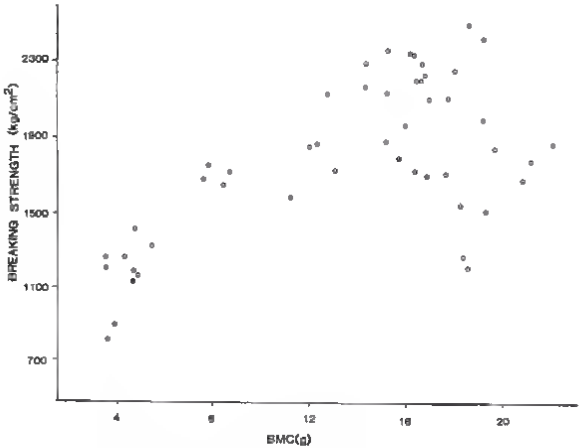

FIGURE 6 RELATIONSHIP BETWEEN BREAKING STRENGTH AND BONE MINERAL CONTENT (BMC) (g/2 cm CROSS-SECTION)

TABLE 2. MOMENT OF INERTIA[1] - MEANS

	IC[2]	I[3]	IE[4]
Means	7.40[a]	3.46[b]	3.55[b]

[a,b]Values with different superscripts are different ($p<.01$).

[1]Moment of inertia (cm^4).

[2]Moment of inertia calculated from circular equation.

[3]Actual moment of inertia computed by SCADS computer program.

[4]Moment of inertia calculated from elliptical equation.

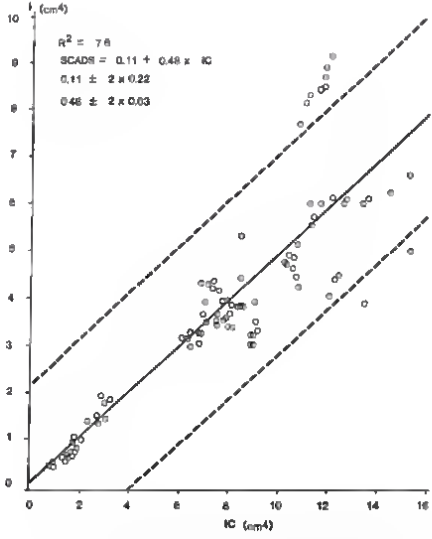

FIGURE 7 RELATIONSHIP BETWEEN THE MOMENT OF INERTIA COMPUTED BY THE SCADS COMPUTER PROGRAM (I) AND A CIRCULAR ESTIMATE OF THE MOMENT OF INERTIA (IC) DASHED LINES REPRESENT 95% CONFIDENCE INTERVAL. REGRESSION EQUATION AND STANDARD ERRORS ARE REPORTED.

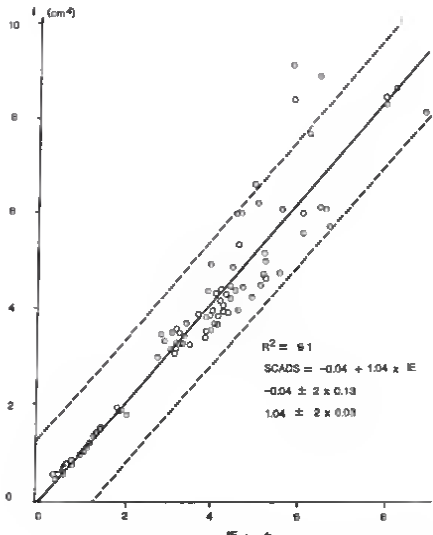

FIGURE 8. RELATIONSHIP BETWEEN THE MOMENT OF INERTIA COMPUTED BY THE SCADS COMPUTER PROGRAM (I) AND AN ELLIPTICAL ESTIMATE OF THE MOMENT OF INERTIA IE.

determine the material properties of bone, and can be used to account for the irregular shape of bones

Breaking load is a measure of the force required to break a bone so total BMC becomes the most critical factor (Crenshaw et al., 1981). The correlation between BMC and breaking load (figure 9) was .961 ($p<.001$) (table 2). When optimum mineralization is achieved, breaking strength is maximized. As bone develops, mineral is deposited within the protein matrix in a highly organized manner and in a specific ratio of mineral to protein. Optimum mineralization produces the lightest (smallest cortical area) bone necessary to provide effective support for locomotion for the animal. However, through dietary manipulation and training programs designed for controlled stress on bone the breaking load may possibly be increased beyond the value due to optimum mineralization by addition of more total bone. Increasing the area of cortical bone over which forces are applied as a horse performs may increase its ability to withstand greater loads during exercise and training.

From the force-deformation curve (figure 10) the breaking load or force required to break the bone is determined by the height of the curve. Horses age 3 to 4 resisted the highest loads before failure. The slope of the curve represents the elastic modulus. The greater the slope, the stiffer the bone. Higher moduli of elasticity were found in older horses. Younger horses had lower moduli of elasticity (i.e. slopes) and showed greater deformations before failure.

In this study the elastic modulus or stiffness of the bone increased dramatically in the first yr of life (10,000 kg/cm^2; 980

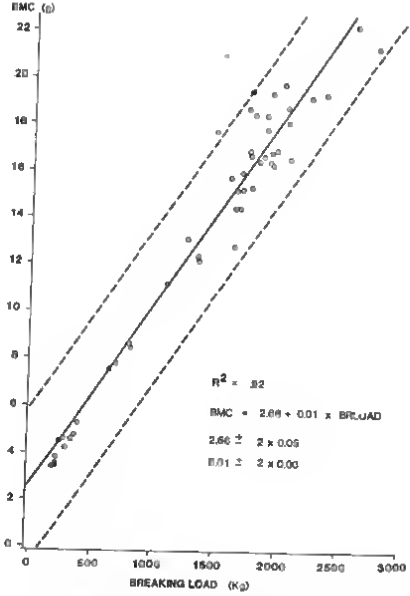

FIGURE 9 RELATIONSHIP OF BONE MINERAL CONTENT (BMC) OF A 2 cm CROSS-SECTION WITH BREAKING LOAD DETERMINED BY 3 POINT BENDING OF EQUINE METACARPALS.

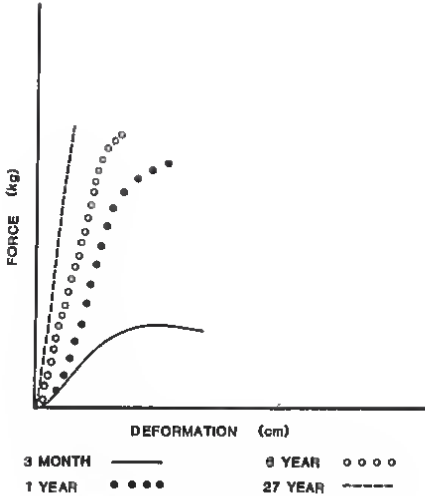

FIGURE 10: FORCE-DEFORMATION CURVES FOR METACARPALS FROM HORSES OF VARIOUS AGES.

MPa) and reached maximum values (37,000 kg/cm^2; 3.6 GPa) by 4 yr of age. From 4 to 27 yr the stiffness of the bone did not change significantly figure 10.

Bynum et al. (1971) reported an average elastic modulus of 119,510 kg/cm^2 (12 GPa) Schryver (1978) reported an average modulus of elasticity of 183,600 kg/cm^2 (18 GPa). Although the same trends were noted in this study and that of Bynum et al. (1971) values reported here are much lower than those reported previously. Possible factors which may affect these differences are rate of loading, length between the supports the bone rests on and the point of application of the load. Bynum et al., (1971) loaded the metacarpals on the anterior surface. In this study the bones were loaded on the posterior surface because less damage occured at the support collars when bones were loaded anteriorly.

Recent research has provided information on forces involved in equine locomotion. Bertel et al. (1978) developed an analytical model of the equine fore digit and the forces created at various gaits. Cheney et al. (1973) determined the influence of track surface on the forces involved in equine locomotion. Information on forces, equine limbs are subjected to in addition to evidence that exercise effects bone strength (Schryver, 1978) increases the importance of biomechanical studies in the equine. The process of bone development dictates stated that only by taking age into account in the adoption of standards of normality is it possible to assess the effect of other factors which may influence bone development (Meema and Meema, 1981)

This study provides an a comparative basis information about age, BMC and the mechanical properties of bone. Continuing studies may

provide information on the relationship of the mechanical properties of bone to growth, development and remodeling in response to exercise and training

CHAPTER III
THE USE OF NON-INVASIVE TECHNIQUES TO PREDICT BONE MINERAL CONTENT AND STRENGTH IN THE HORSE

Radiographic Photometry

For over 20 years researchers have sought improved methods for predicting bone mineral content (BMC) and skeletal strength (Mason and Rutves, 1965; Cameron and Sorensen, 1963, Abendschein and Hyatt, 1970) The commercial availability of non-invasive techniques for evaluating bone has created interest within the horse industry and increased the demand for information on the predictive abilities of the various methods. Presently three non-invasive techniques for predicting BMC and/or breaking strength are in varying phases of development for use in the equine. Photon absorption, radiographic photometry and ultrasound are the techniques receiving the most interest for the horse, with each technique having advantages and disadvantages

Early reports of the use of conventional radiography to evaluate the mineral content of bone indicated that a minimum of 30% and possibly up to 50 or 60% loss of mineral had to occur before a cavitation could be detected visually (Ardran, 1951; Lachman, 1955). In the late 1940's an objective technique for the in vivo quantitative evaluation of the mineralization of bone was developed at Pennsylvania State College, School of Chemistry and Physics. This pioneering work of Mack et al. (1949) led to the development of the Pennsylvania State Bone Density Research and Evaluation Center.

Brown and Buckley (1951) working at Pennsylvania State designed an apparatus for evaluation of bone that was based on the principle that the attenuation or absorption of X-radiation as it passes through bone and, therefore, the amount of light passing through a radiograph is related to the mineral content of the bone. The authors stated that by use of an ivory stepwedge as a standard, estimates of BMC could be obtained.

McFarland (1954), using the apparatus described by Brown et al. (1951), conducted a survey study in which 1200 people of all ages and both sexes were evaluated radiographically. They reported BMC values of males to be approximately twice that of females. McFarland (1954) found the technique to be very reproducible with a variability of 5%.

Ekman et al. (1970) reported a coefficient of variance of 4.1% after 12 determinations of the percent bone mineral were made on a foreleg of a pig. Meema et al. (1972) studied 32 ambulatory patients who suffered from terminal chronic renal disease, on histological examination the incidence of bone disease was 84% while radiological evidence of bone loss was found in 73% of the examinations.

In order to make direct photodensitometric comparisons between X-rays a reference standard must be exposed simultaneously with the bone. McFarland (1954) employed the use of an aluminum stepwedge. Aluminum has an atomic number close to that of hydroxyapatite and is more homogenous than ivory. The radiological correlation between hydroxyapatite and aluminum improves as energy levels increase (Meema et al., 1964). Cassell (1957) stated X-rays are absorbed by aluminum to the same degree as bone mineral, therefore, results can be expressed in millimeters of aluminum or bone mineral content.

The use of an aluminum stepwedge as a standard has received some criticism. Pridie (1967) stated standards made of materials other than bone produce different absorption curves which may vary with the composition of the standard material and with energy levels of the X-rays. Meema et al. (1964) used a dipotassium hydrogen phosphate standard. They reported an effective atomic number of 15.59 for K_2HPO_4 and 15.86 for hydroxapatite and suggested this standard is more appropriate than aluminum with an atomic number of 13. Despite criticisms aluminum stepwedges have been widely used with good results (Andersen et al., 1966, Meskin et al., 1981).

Many of the errors in radiographic photometry are those inherent in the use of X-ray. These include variation in energies of X-ray beams which cause nonuniformity in film exposure and radiation scattering. The "heel effect" was demonstrated by exposing only a film thus assessing the film on a densitometer (Andersen et al., 1966). There was a gradient of radiation intensity parallel to the long axis of the tube, and the intensity was constant at right angles to this direction. Pridie (1967) reported that reducing the size of the field by adjusting the film focus distance can reduce the nonuniformity of radiation to negligible proportions.

Intensifying screens are also a potential source of error in radiographic photometry. Andersen et al. (1966) stated that better definition is obtained without screens and that screens may have local variations in their response. Meema et al. (1964) stated that cassette with intensifying screens provide a source of irregular densities. They reported that there were areas of equal density that could be identified

and used for bone density measurements. Ekman et al. (1970) found it was not possible to obtain sufficient density without intensifying screens at low energies. They selected screens from the same emulsion number and tested them. The benefits obtained in lowered radiation hazards by use of screens outweighs the disadvantages of possible errors (Ekman et al., 1970). Meskin et al. (1981) found high correlations between bone mineral and radiographic bone aluminum equivalents (BMAE) with low coefficient of variations using intensifying screens at a voltage of 57 kv and an amperage of 30 ma with a focal film distance of 91.5 cm.

Soft tissue covering introduces error in bone density estimations (Mazess et al., 1964). Mazess et al. (1964) choose the human radius as a site for radiological measurement. To account for soft tissue effects the arm was submerged in water which has nearly the same absorption coefficient as soft tissue. Despite this effort to account for soft tissue effects the authors reported that when radiographs were made through soft tissue they were unable to detect osteoporosis in two cases where significant demineralization had taken place. When X-rays were taken of excised bone the demineralization was evident. Anderson et al. (1966) X-rayed an aluminum stepwedge in water and upon densitometric analysis found that the thickest step on the wedge appeared to absorb less radiation than the next smaller step.

To avoid errors created by soft tissue, researchers have used the phalanges where soft tissue covering is minimal (McFarland et al., 1951, Anderson et al., 1966). Most radiologic determination of bone mineral have been limited to the cortical area of long bones. Mazess et al. (1964) listed two reasons for this: 1) trabecular bone is always

surrounded by a cortex of variable thickness and 2) there is wide variation in trabecular mineralization. Although cancellous bone is more labile if bone turnover is caused by diet or hormonal factors, these factors will probably effect the skeleton as a whole and a sensitive technique should be able to detect changes.

Meskin et al. (1981) correlated RBAE, determined by radiographic photometry, with BMC on 17 equine fusulage from horses of varying breeds and ages. They reported correlations of RBAE with BMC of .94, .88 and .92 for the selected points, lateral and medial cortical aspects and the midpoint. Meskin et al. (1981) reported coefficient of variation ranging from 5.02 to 1.3%.

When careful attention to potential sources of errors are considered and minimized, the advantages of a short exposure time and the fact that the entire metacarpal can be X-rayed make the use of radiographic photometry an attractive technique for study of bone development in the equine.

Radiography also offers the advantage of yielding morphometric measurements of cortical bone. These simple measurements are easy to perform, inexpensive, can be standardized and provide valuable information about bone mass. Total mass of bone is the sum of trabecular and cortical bone. However, since cortical bone comprises 80% of the skeleton, information on cortical bone should reflect overall skeletal status (Mazess et al., 1972; Mazess and Mazess, 1981).

Takahashi and Frost (1966) stated that, in general, mature bone may be gained or lost at one or any combination of four locations: the periosteal surfaces, the surfaces of vascular channels within cortical

bone, the cortical endosteal surface and the trabecular-endosteal surface. They suggest that if a normal population is defined by "physiologic" age then by exposition weakened or diseased bone may be identified. Large scale surveys in the 1960's documented significant bone loss in women over 50 with measurements of cortical thickness of the radius and the second metacarpal (Garn et al., 1964, Exton-Smith et al., 1969, Meema et al., 1972).

To estimate bone mass from anteroposterior radiographs of long bones with a cylindrical geometry the equation $a^2 - b^2$ (figure 3) provides an estimate of cross-sectional cortical area (Exton-Smith et al., 1969). If the cross-section more closely resembles an elliptical geometry then a lateromedial radiograph must be taken to obtain values for the equation $AD - bd$ (figure 3).

Despite the imperfect geometry of bone and variations in the composition of compact bone due to intracortical porosity and mineralization, correlations of radiographic measurements and bone mineral content have been good (Meema and Meema, 1981). Exton-Smith et al. (1969) reported a high correlation between cortical area calculated by $a^2 + b^2$ (figure 3) and metacarpal bone ash ($r = .85$). Neuman and Kichy (1972) correlated combined cortical thickness and cross-sectional cortical area with midmetacarpal ash content per unit length and obtained correlations of .68 and .78, respectively.

El Sherif et al. (1979) studied the relationship of ash and cortical area determined by planimetric measurements of cross sections of equine third metacarpals. They reported correlations of .63. Meakin et al. (1981) compared circular and elliptical cross-sectional indices

calculated by measurements from radiographs of equine third metacarpals with BMC. They reported correlations of .95 for the elliptical area index and .88 for the circular area index.

Studies of growth rates of Thoroughbreds and Quarter Horses have included cannon bone circumference as an indicator of skeletal growth (Hintz et al., 1979, Gentry et al., 1983). Bone develops in a manner such that metacarpal circumference increases with age regardless of the overall skeletal status (Meyers and Meyers, 1981). In order to accurately estimate bone mass, changes occurring at the endosteal surface must be determined. Radiography offers the advantage of providing morphometric measurements for estimating changes in cortical area.

Photon Absorptiometry

Mason and Ruthven (1965) developed an X-ray bone densitometer that represents the link between X-ray densitometry and photon absorptiometry. Both methods depend on the principle that attenuated radiation is proportional to bone mineral. Mason and Ruthven were able to obtain greater precision in the X-ray technique by reducing scatter of the X-ray beam through collimation. The densitometer used low-intensity X-rays and a scintillation detector coupled with a photomultiplier tube. The beam was scanned and linear absorption curves were plotted directly on an X-Y recorder.

Cameron and Sorenson (1963) described an improved method for measuring BMC in vivo by photon absorptiometry. The authors stated that photon absorption was different from X-ray densitometry in the following ways: 1) the transmission of the photon beam is measured directly by a scintillation detector system, 2) the photon beam is monochromatic, 3)

the effects of the tissue around the bone are accounted for. Cameron and Sorenson (1963) state that these factors eliminate many errors inherent with the use of densitometry including these due to variability of X-ray films and development, errors due to scattered radiation, uncertainties in absorption coefficients and tissue effects.

As bone is scanned in a rectilinear pattern photon radiation is either transmitted, scattered or absorbed. The concentration of bone mineral and soft tissue in the path of the radiation beam determines the number of photons reaching the detector.

Cameron and Sorenson (1963) described the housing apparatus for the collimated photon source as a stainless steel tube 1 cm long, 3 cm in diameter. The tube is placed in a lead cube 5 cm below the photon surface with only a 3 cm opening for collimating the radiation. The source is rigidly coupled to a NaI crystal scintillation detector system. The source and detector simultaneously scan the bone transverse to the long axis.

In monochromatic photon absorption soft tissue effects are accounted for by placing a water filled bag or water bath around the limb. Water and soft tissue have similar absorption patterns. Because the depth of the water bath is uniform, the absorption due to soft tissue and water remain constant and serve as a baseline. Subtraction of the baseline values from total absorption results in absorption due to bone (Meyers et al., 1979).

The emission of two separate photon energies in dual photon absorptiometry permits this system to discriminate between soft tissue and bone mineral and to calculate the quantities of each present.

Radionuclide sources used in dual photon absorptiometry techniques are ^{125}I/^{241}Am, ^{241}Am/^{137}Cs or radionuclides that emit at two discrete energies; ^{153}Gd or ^{109}Cd. The use of dual photon absorptiometry eliminates the necessity of surrounding the bone with a tissue equivalent. Areas of the body such as hips, spine or the entire body can be scanned with this system (Mazess, 1981).

As the bone is scanned, the number of photons reaching the detector can be plotted in a transmittance curve. The area of this curve is proportional to the BMC (Smith et al., 1972). Although a graphic solution can be used, most systems are now coupled to microcomputers and BMC is calculated by a mathematical relationship (Wentworth et al., 1971). Standards that simulate bone have been developed to calibrate the system and serve as a reference guide for BMC calculations (Mazess, 1981).

The estimated bone mineral values generated by photon absorptiometry are BMC, BMC/bone width (W) and paradensity (Stalikier et al., 1976). BMC (g/cm) is the bone mineral contained in a cross-section of bone 1 cm in length. The BMC/W is a calculated value which adjust BMC for bone and body size. The paradensity index is calculated by dividing BMC by the cross-sectional area (g/cm^3). Paradensity is similar to specific gravity or ash content. The calculated paradensity assumes that the cross-sectional area is a circle and makes no adjustment for the marrow cavity or trabecular and cortical bone. Although the relative value of these indices has not been determined much of the reported research has used BMC (Meyer et al., 1968; Wentworth et al., 1971, Stalikier et al., 1976; Myers et al., 1979).

Cameron and Sorenson (1963) initially validated the photon absorption technique by testing blocks of paraffin containing $CaCO_3$, homogeneously mixed in known proportions. The weight of $CaCO_3$ was tested over a wide range of concentrations, to an accuracy of within 3%.

Meyer et al. (1968) tested the reproducibility of the photon absorption technique on chickens. They measured a standard (aluminated lead tube mounted on a plastic holder) and 5 eighteen m old male birds 9 times in 14 d period. The calculated BMC values for standards had a coefficient of variation of 2.6%. The BMC values for the males varied in a cyclic manner from day to day. The authors suggested the fluctuations may have been due to variations in daily feed intake and may be important in short-term mobilization for mineral or ionic balance. These results suggest a high degree of sensitivity for this technique.

Smith et al. (1972) made 21 repetitive BMC measurements on a single normal human subject over a 2 wk period and reported a coefficient of variation of 2.5% for cortical bone and 2.7% for trabecular bone. Sanchez et al. (1981) found coefficient of variations for BMC/W of 2.2 to 6.6% for 10 measurements on six rats. They reported correlations between BMC/W on whole femur ash weight and dry weight of .93 and .96.

The photon absorption technique was developed as a tool to aid research in the area of osteoporosis of elderly women. Cameron and Sorenson (1963) scanned 137 human females and clearly identified a group over 50 years of age with diagnosed osteoporosis. Repeat measurements on subjects were reproducible within 3%.

Meyer et al. (1968) adapted the Cameron-Sorenson technique for in vivo determination of BMC for use in poultry. They found a correlation between bone mass and ash weight per cm bone of .95. The bone mass determination were reproducible within 3.2%.

Myers et al. (1979) evaluated photon absorptiometry using the rabbit as a model for the study of bone mineral changes. The authors found high correlations between bone mineral mass and age (r = .88, male, r = .81, female) and body weight (r = .72, male, r = .81, female).

Sanchez et al. (1981) also reported positive correlations between body weight and femur BMC/W (r = .93) in rats. BMC/W determined by photon absorptiometry was also correlated with whole femur ash weight (r = .93) and dry weight (r = .93).

Wentworth et al. (1971) designed a photon absorption apparatus with breakaway source and detector for use on large animals. The photon source was interchangeable, ^{125}I for dogs and smaller animals, ^{241}Am was used for equine bones. Correlation coefficients for bone ash (g/cm) and canine and equine bone was .99. Wentworth et al. (1971) states that to perform a scan on mature dogs and horses anesthesia was necessary to assure immobilization. A group of 16 Beagles 1 yr of age was fed a diet containing 0.12% Ca and 1.2% P for 42 wk. After this Ca depletion phase they were given a diet containing 1.0% Ca and 0.8% P. The mandibles were removed from dogs sacrificed at 4, 8, 12, 16, 20 and 28 wk and scanned. After beginning the repletion diet a progressive increase in BMC was dependent on the number of wk of feeding the Ca repletion diet (P<.01) (Wentworth et al., 1971).

Photon absorptiometry was performed on mandibles of adult beagles fed a low-Ca high-P diet for 41 wk then supplemented with varying levels

of fluoride (Henrikson et al., 1970). Results showed that mineral mass decreased with increasing levels of fluoride. Radiographic and microradiographic examination of the humerus, femur and teeth failed to demonstrate an effect of added fluoride. Henrikson et al. (1970) explained the incongruence between the results by suggesting that the bone of the mandible, composed mainly of trabecular bone has the highest rate of turnover and is, therefore, more sensitive to short term effects.

Krook et al. (1971) depleated bone Ca in a group of adult beagles using a diet containing 0.12% Ca and 1.2% P. Nutritional osteoporosis was diagnosed in the dogs and after 42 weeks on the low Ca diet. The animals were placed on a repletion diet with 1.0% Ca and 0.8% P. Photon absorptiometry showed remineralization was a linear function of time on the repletion diet and was completed after 24 weeks.

Martin et al. (1981) performed a survey study with 154 beagles (79 M, 74 F) ranging in age from 14 to 187 m. BMC, bone width and BMC/W of the right tibia were determined using photon absorption. Weight had a linear effect on BMC (R^2 = .61) and BMC/W (R^2 = .41) for males and females. Peak BMC and BMC/W were reached at six yr of age at which time they began to decline.

Jeffcott (1985) used photon absorptiometry (Norland Digital Bone Densitometer[1]) to assess bone quality in 27 thoroughbred or crossbred horses ranging in age from less than 1 wk old to 16 yr of age. Metacarpals from equine cadavers showed rapid increases in BMC during

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[1]Prototype Model 2781. Large Animal Scanning System (LASS): Norland Corporation, Fort Atkinson, WI

the first 6 m followed by a more gradual increase in BMC up to 7 to 8 yr and followed by some decline as the animals neared 15 yr of age.

Jeffcott (1985) also reported on the results of partial weight-lessness on BMC in the horse. A 3 year old mare was confined in a stall for two wk then transferred to a flotation chamber where body weight was decreased by 75%. The mare was then returned to a stall for 2 wk followed by 1 wk of free exercise in a small paddock along with 30 min forced walking and trotting exercise. The photon absorption evaluation showed a drop in BMC from 9.31 to 8.22 g/cm in the first wk with a continued decline in 7.42 g/cm at 14 d. Exercise brought about remineralization of the bone and BMC values returned to 8.22 g/cm.

Jeffcott (1985) reported that photon absorption is a repeatable and reliable technique but noted several disadvantages: 1) scanning time of 90 seconds, 2) scanning orientation, 3) the use of water-filled bandages, and 4) failure to account for bone geometry.

Photon absorption is a highly sensitive and accurate in vivo method for estimating BMC and in a controlled research environment provides an excellent tool for monitoring changes in bone mass.

Ultrasound

Ultrasound was developed in the 1920's, when sonar made it possible for ships to locate submarines within their transmitting area. Water acted as a sound transporting medium for high frequency sound waves which could detect objects with densities different from the transporting medium.

The use of ultrasound as a diagnostic tool in the horse was first developed by Pratt (1980). Pratt (1980) found that the velocity of sound traveling through bone reflected the strength and weakness of

equine cannon bones. Pratt (1982) described the velocity as an "effective" velocity. The actual path the sound waves take through the bone has not been determined. The "effective" velocity is determined by placing transducers on either side of the bone that transmit and receive sound waves created by an ultrasonic generator. The time it takes the sound wave to be initiated at one transducer, travel through the bone and be picked up at the opposite transducer (t) is divided into the width of the cannon bone (d) predetermined by vernier calipers. This calculation is the estimated velocity (v) of sound through bone (v = d/t).

Soundwaves travel through bone with a velocity (m/sec) that represents the material and structural properties of bone (Gerlach et al, 1975). Ahendschein and Hyatt (1972) defined the relationship between ultrasound velocity and bone. The authors found a linear correlation between the modulus of elasticity determined by testing guinea pig femurs loaded in compression and the ultrasound velocity. The ultrasound velocity was used in the following equation for calculation of the modulus of elasticity $E = V^2D$, where E = modulus of elasticity, V is velocity of ultrasound and D is the mass density. Ahendschein and Hyatt (1972) state that correlations between the modulus of elasticity and breaking strength, suggest a direct relationship. Therefore, there appears to be a clear relationship between the physical properties of the modulus of elasticity, and breaking strength of bone and the velocity of sound through bone (Ahendschein and Hyatt, 1972, Greenfield et al., 1975).

Ultrasound also reflects certain chemical properties of bone. Rich et al. (1966) measured the velocity of ultrasound transmission through rectangles of fresh bovine cortical bone of varying size. The correlation between mass of calcium per cm^2 and ultrasound velocity was .99.

Greenfield et al. (1961) used ultrasound and photon absorptiometry to measure the velocity of sound and BMC in the human radius. The authors used these measurements and a bone model to calculate bone mineral density, compact bone density and modulus of elasticity. Measurements were made in vivo so actual determinations of bone parameters could not be reported but all measurements compared favorably with the literature. To test the clinical efficacy of their method they compared BMC and E determined by ultrasound and photon absorption with several currently available methods for predicting bone loss. Greenfield et al. (1961) reported the ultrasound-photon absorption technique was a more accurate predictor of osteoporosis than a visual radiographic score, plasma alkaline phosphatase and/or serum creatinine.

Garlanc et al. (1975) made ultrasonic velocity measurements of 666 human males and females. The data demonstrated a pattern of decreasing bone density with age which corresponds with the findings of other studies. They were also able to accurately follow the healing of fractures by sequential measurements using the unfractured limb as a standard.

Pratt et al. (1962) was able to monitor the healing of non-union scaphoid fractures in the horse with the use of ultrasound. In one case, radiographs revealed a non-union scaphoid fracture, so ultrasonic

pulse was able to be detected for 14 d. The first transmitted velocity through the fractured bone was 1760 m/sec. The velocity through the unfractured sesamoids on the opposite metacarpal was 2035 m/sec. Over a 108 d period the velocity of sound through the fractured sesamoids increased to 2315 m/sec while that through the opposite leg was 2346 m/sec.

Glade and Lobo (1984) followed the development of equine cannon bones in Thoroughbreds between 8 and 12 m of age. The animals were fed either 100 or 130% of the NRC (1978) energy and protein recommendation. Glade and Lobo (1984) reported that bone strength decreased linearly in all horses an average of .021 mm/d. Bone strength was calculated by an equation relating soft tissue corrected sound velocity, bone density and bone breaking strength. The decrease in breaking strength reported by Glade and Lobo (1984) is not consistent with the literature (Symon et al., 1971; Rabin et al., 1983).

Rabin et al. (1983) reported ultrasound data collected by the Association for the Advancement of Sports Potential, Equine Division. The information was collected on 262 thoroughbreds in training or major races. They reported their accuracy to be better than 1% and reproducibility to be better than 0.5%. They report a correlation coefficient of .48 between the left and right metacarpals. A high correlation was also reported for ultrasound velocity and age 1 through 3 yr (r = .87). However, when readings from 4 and 5 yr of age were included the correlation coefficient dropped to .78. Rabin et al. (1983) also presented several clinical cases where ultrasound was used to predict or monitor changes in bone strength due to training related

injury. In conclusion the authors state that the sequential evaluation for asymmetry between ultrasound determination on both legs is beneficial to the clinical determination of fracture threshold.

Jeffrott and McCartney (1985) compared ultrasound velocities from 18 normal equine cadaver metacarpals with specific gravities. The authors reported an apparent linear relationship but noted considerable variation in ultrasound velocities according to age. Regressing analysis of velocity versus age in horses over 1 yr old showed a linear relationship with variances about the line of ± 61 m/sec. Standard deviations for individual points were ± 20 m/sec. The reported the technique was simple, inexpensive and reliable. The authors also noted that behavioral problems associated with performing the procedure were minimal and easily overcome.

Evaluation of Equine BMC and Bone Strength

A study was designed to evaluate the predictive capabilities of photon absorptiometry, radiographic photometry and ultrasound comparing the techniques on a common group of equine third metacarpals with BMC determined by ashing and bone strength determined by flexure test.

Experimental Procedure

The BMC of 25 pairs of third metacarpals (McIII) was estimated by three non-invasive techniques, radiographic photometry, ultrasound and photon absorptiometry. The right and left McIII of equine cadavers of varying breeds and ranging in age from 1 d to 27 yr were used. Anteroposterior radiographs of McIII were taken with an aluminum stepwedge exposed simultaneously as a reference standard. A photometric area of the stepwedge and bone (1 cm below the nutrient foramen)

provided an estimate of BMC in radiographic bone aluminum equivalents (RBAE) (Meakim et al., 1981). After radiographs were taken all tissues were removed and McIII were wrapped in .9% saline soaked gauze, placed in plastic bags and frozen at -15°C until further analysis.

High frequency ultrasound was used to evaluate BMC and bone strength by measuring time elapsed and distance from one transducer placed on the lateral side of McIII and a second transducer placed on the medial side. Measurements were made at the mid-point and 1 cm below the nutrient foramen of the bone. Sound waves travel through bone with a velocity that represents the material and structural properties of bone (Gerlach, et al., 1975). The ultrasonic generator was a Panametrics 5055 transmitter/receiver[1] which was coupled to KB Aerotech 1MHz matched transducers. The transducer signal was displayed on a Hewlett Packard oscilloscope[2]. Calipers were used to measure latero-medial diameters of bone.

A Lunar Radiation Corporation[3] analyzer was used for dual photon absorptiometry which included a collimated radionuclide source emitting a radiation beam at two separate energies. As the McIII were scanned, radiation was transmitted, scattered or absorbed depending upon the amount of mineral in the bone. Dual photon absorptiometry is designed to account for soft tissue effects by using gamma rays at two separate energies. McIII were embalmed in a .9% saline solution which has a

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[1]Panametrics. Model 5055. Waltham, MA.

[2]Ballantine Lab Incorporated. Model 1023AB. Boonton, NJ.

[3]Lunar Radiation Corporation. Model DPC. Madison, WI.

similar absorption pattern to soft tissues. The scan line is .35 cm in width, six lines were averaged at the midpoint and BMC is reported in grams per 2.1 cm section.

Wet McIII were tested by the point bending on a testing machine[1] at a deformation rate of 50 mm/min. Broken bones were reassembled and 2 cm sections were removed at the point of break and 1 cm below the nutrient foramen. These cross-sections were photographed then digitized using a microcomputer with a graphics tablet. The SCADS computer program was used to compute the cortical area and the moment of inertia without assuming geometrical shape (Piotrowski et al., 1983). Caliper cross-sectional measurements from bones and radiographs were determined for AP bone diameter (B), AP medullary diameter (b), Intermediol (LM) bone diameter (D), and LM medullary diameter (d) (figure 2). Equations for calculating area indices were circular cross-sectional area index (CAI) from bone and radiograph XAI ($B^2 - b^2$) and elliptical cross-sectional area index (EAI) from bone ($BD - bd$).

Preparation of 2 cm cross-sections involved resting metacarpals in containers made to conform to the size of the bone. Liquid acetone[1] which solidifies, was poured around the metacarpals to provide a uniform flat surface so 2 cm cross-sections could be accurately cut from bones. Cross-sections not conforming to 2 cm length as determined by vernier calipers were ground until proper specifications were attained. The bone sections were ether extracted then ashed at 600C for 12 hrs. Bone ash was expressed as grams per 2 cm section and defined as BMC.

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[1]Instron Corporation. Canton, MA.

[2]Sand foam. Ante Marine Supply, Inc., Pinellas Park, FL.

Results and Discussion

BMC increased with age from 1 d to 3 yr (figure 11 and figure 12). Figure 11 represents BMC from bone sections removed 1 cm below the nutrient foramen and figure 12 represents BMC from the point of the break. No differences were found due to right and left McIII or sex. Most of the mineralization occurred during the first year, BMC were 3 to 4 g for newborns and increased to 14 to 16 g for yearlings. There was a more gradual increase in BMC up to 3 to 4 yr when BMC reached the highest values. Between 13 to 27 yr of age there was a tendency for BMC to decline. These results are similar to the work of El Shorafa et al. (1979).

As BMC increased, breaking strength increased in an exponential fashion (figure 6) similar to the response reported by Vane and Kubela (1959). Curry (1969) explained the failure of bone to completely mineralize and then produce a linear relationship between BMC and breaking strength by suggesting that mineralization beyond an optimum value would decrease resiliency of bone and reduce its ability to resist dynamic loading. Breaking strength (kg/cm^2) reflects the force required to break the bone and the distribution of the area over which the force was applied. The correlation between BMC and breaking strength was .643 ($p<.0001$) (table 2).

Breaking load is the force (kg) required to break a bone. Breaking load is determined by the size and quality of the osseous material. The correlation between BMC and breaking load (figure 9) was .961 ($p<.0001$) (table 2). The correlations between equations for calculating area indices and BCADS are shown in table 3. In figures 13, 14 and 15 the

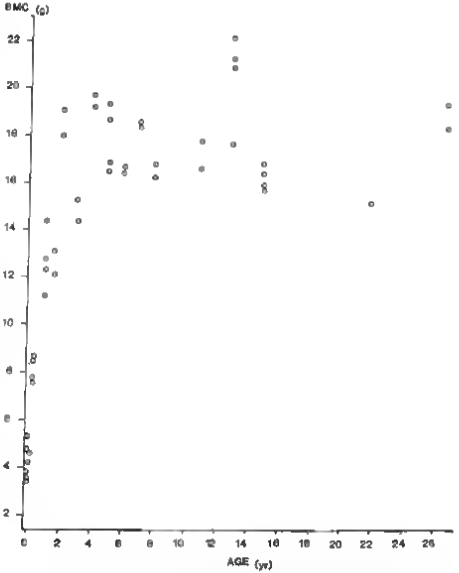

FIGURE 11. RELATIONSHIP BETWEEN BONE MINERAL CONTENT (BMC) OF 2 cm CROSS-SECTION REMOVED FROM 1 cm BELOW THE NUTRIENT FORAMEN OF METACARPALS AND AGE OF HORSES.

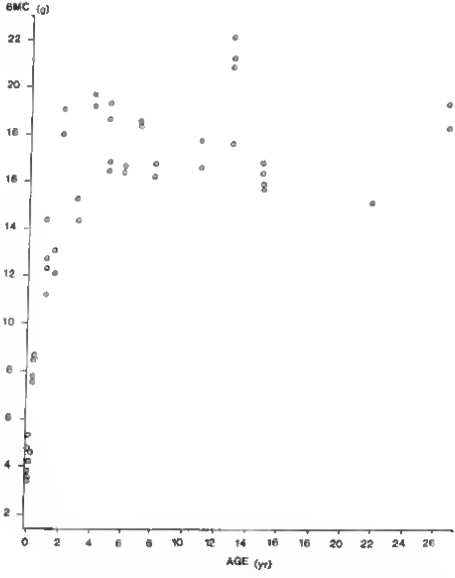

FIGURE 12. RELATIONSHIP BETWEEN BONE MINERAL CONTENT (BMC) OF 2 cm CROSS-SECTION REMOVED FROM THE BREAK POINT OF METACARPALS AND AGE OF HORSES.

TABLE 3. CORRELATION COEFFICIENTS FOR SCADS VS AREA INDICES

	XAI[1]	CAI[2]	EAI[3]
SCADS ($p<.0001$)	.893	.957	.979

[1]Radiographic area index.

[2]Circular area index.

[3]Elliptical area index.

[4]Computer generated area

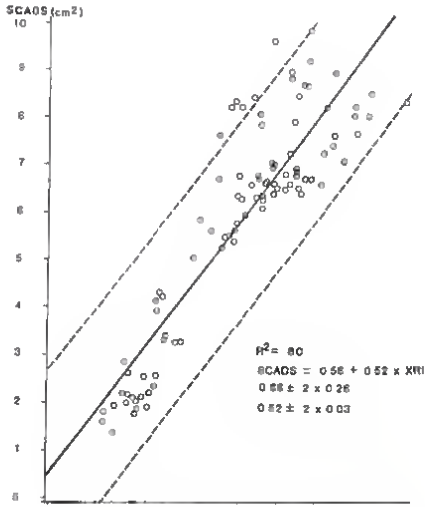

FIGURE 13. RELATIONSHIP BETWEEN ACTUAL COMPUTED CORTICAL AREA (SCADS) AND RADIOGRAPHIC CORTICAL AREA INDEX (XRI) CALCULATED WITH CALIPER MEASUREMENTS FROM RADIOGRAPHS.

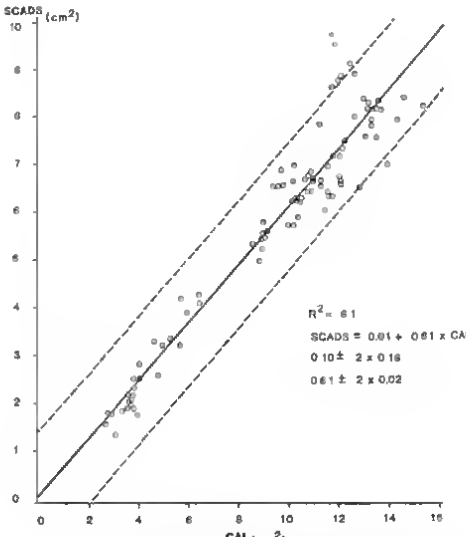

FIGURE 14. RELATIONSHIP BETWEEN ACTUAL COMPUTER CORTICAL AREA (SCADS) AND CIRCULAR CORTICAL AREA INDEX CALCULATED WITH CALIPER MEASUREMENTS FROM BONE CROSS-SECTIONS.

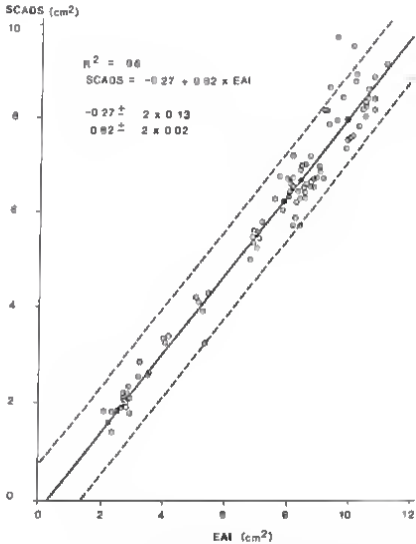

FIGURE 15. RELATIONSHIP BETWEEN ACTUAL COMPUTED CORTICAL AREA (SCADS) AND ELLIPTICAL CORTICAL AREA INDEX CALCULATED WITH CALIPER MEASUREMENTS FROM BONE CROSS-SECTIONS

linear relationships between the area indices and the actual area determined by SCADS are compared. These results suggest that the EAI best approximates the cortical area of Mr111.

If normal equine cortical bone is uniformly mineralized a linear relationship should exist between cortical area and BMC (figures 16, 17, 18 and 19). All area indices were good predictors of BMC (table 4) with EAI and SCADS having the highest correlations and the least scatter of the data.

Radiographic Photometry. Figure 20 represents a plot of BMC and SBAE in mm of aluminum (mm Al) for the medial peak (ML) which corresponds to the longest path through bone on the medial side of the medullary cavity. The correlation between BMC and ML was .948 ($p<.0001$). Figure 21 represents the plot of BMC vs RBAE for the lateral peak (LA), correlation was the lowest of three points .907 ($p<.0001$). The midpoint (CM) RBAE is plotted against BMC in figure 22, $r = .947$ ($p<.0001$). High correlations between BMC and RBAE suggest that prediction equations derived from one or a combination of the plots of BMC vs SBAE may provide an accurate method of estimating BMC. The RBAE from the three selected points were averaged and the estimated SBAE was plotted against actual BMC (figure 23) ($r = .956$, $p<.0001$). The correlations for the average RBAE and breaking strength was .72 ($p<.001$) (table 5).

Larhman (1955) reviewed radiographic diagnosis of osteoporosis and reported that a minimum calcium loss of 30% was necessary before a visual loss could be detected by radiograph. While this statement is true of conventional radiography, radiographic photometry provides a

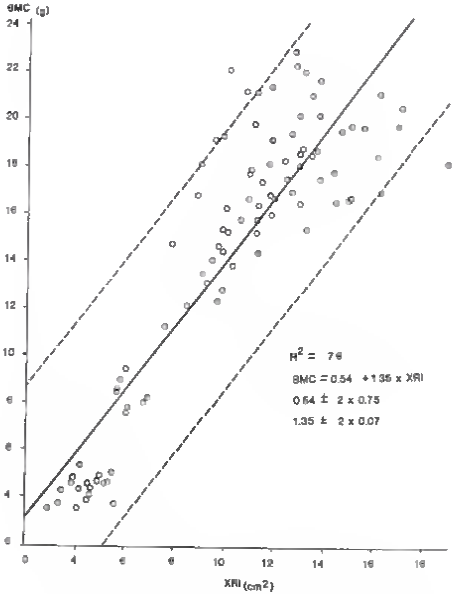

FIGURE 16. RELATIONSHIP BETWEEN BONE MINERAL CONTENT (BMC) OF 2 cm CROSS-SECTION OF EQUINE METACARPAL AND RADIOGRAPHIC CORTICAL AREA INDEX (XRI).

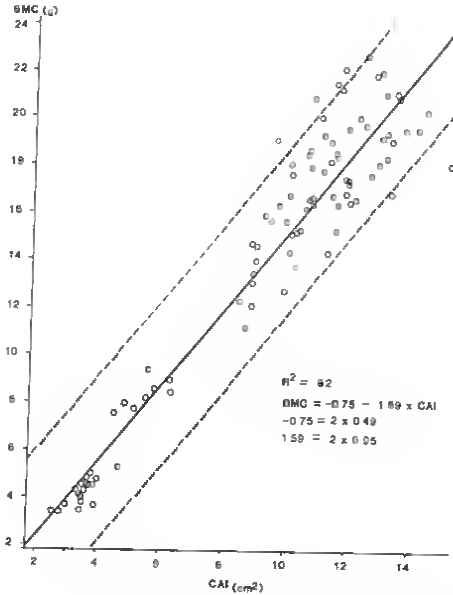

FIGURE 17. RELATIONSHIP BETWEEN BONE MINERAL CONTENT (BMC) OF 2 cm CROSS-SECTION OF EQUINE METACARPAL AND CIRCULAR CORTICAL AREA INDEX (CAI)

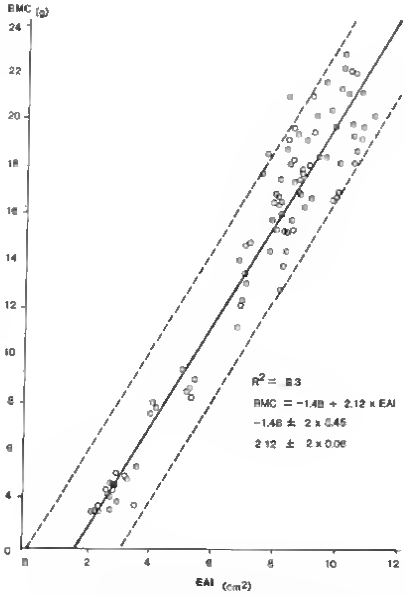

FIGURE 18 RELATIONSHIP BETWEEN BONE MINERAL CONTENT (BMC) OF 2 cm CROSS-SECTION OF EQUINE METACARPAL AND ELLIPTICAL CORTICAL AREA INDEX (EAI).

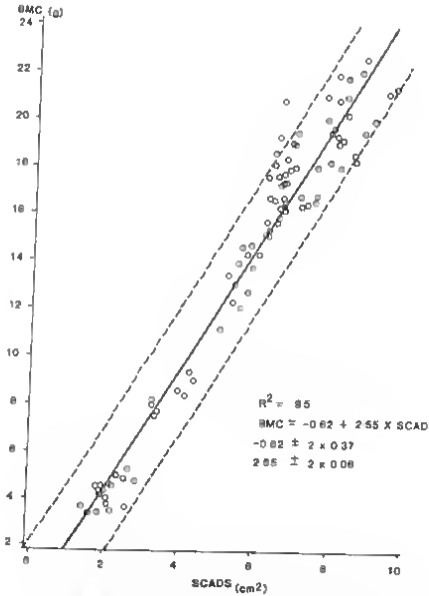

FIGURE 19 RELATIONSHIP BETWEEN BONE MINERAL CONTENT (BMC) OF 2 cm CROSS-SECTION OF EQUINE METACARPAL AND ACTUAL COMPUTED CORTICAL AREA (SCADS).

TABLE 4 CORRELATION COEFFICIENTS FOR BMC VS AREA INDICES

	XAI[1]	CAI[2]	EAI[3]	SCADS[4]
BMC ($p<.0001$)	.890	.957	.966	.974

[1]Radiographic area index.

[2]Circular area index.

[3]Elliptical area index.

[4]Computer generated area.

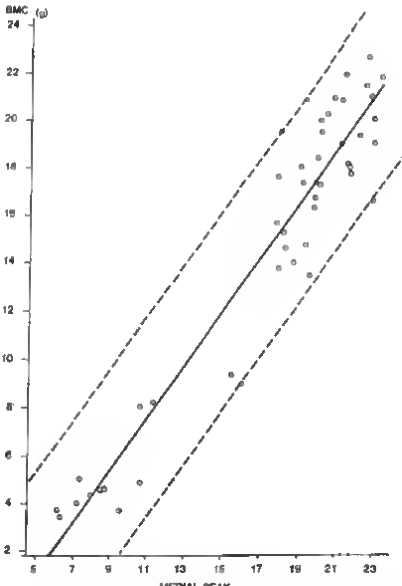

FIGURE 20. RELATIONSHIP BETWEEN BONE MINERAL CONTENT (BMC) OF 2 cm CROSS-SECTION TAKEN FROM EQUINE METACARPALS 1 cm BELOW THE NUTRIENT FORAMEN, AND THE MEDIAL PEAK OF RADIOGRAPHIC BONE ALUMINUM EQUIVALENTS (RBAE) CORRESPONDING TO THAT CROSS-SECTION

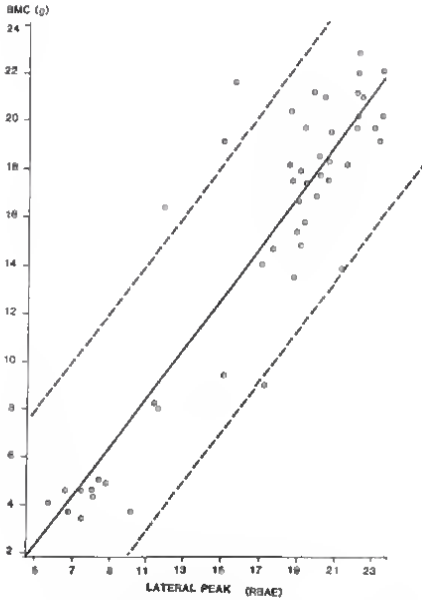

FIGURE 21. RELATIONSHIP BETWEEN BONE MINERAL CONTENT (BMC) OF 2 cm CROSS-SECTION TAKEN FROM EQUINE METACARPAL, 1 cm BELOW THE NUTRIENT FORAMEN, AND LATERAL PEAK OF RADIOGRAPHIC BONE ALUMINUM EQUIVALENTS (RBAE) CORRESPONDING TO THAT CROSS-SECTION

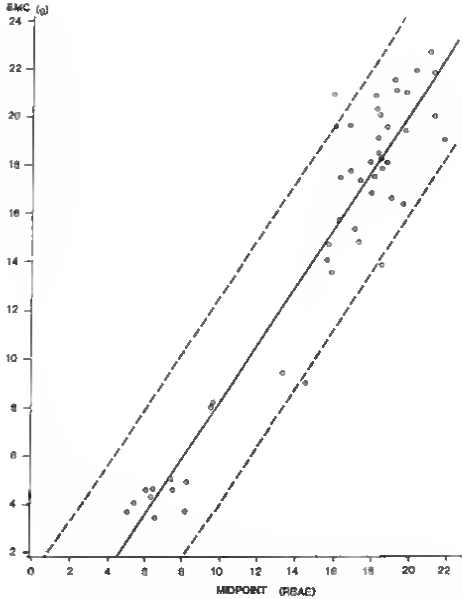

FIGURE 22. RELATIONSHIP BETWEEN BONE MINERAL CONTENT (BMC) OF 2 cm CROSS-SECTION TAKEN FROM EQUINE METACARPAL, 1 cm BELOW THE NUTRIENT FORAMEN, AND MIDPOINT, RADIOGRAPHIC BONE ALUMINUM EQUIVALENTS (RBAE) CORRESPONDING TO THAT CROSS-SECTION.

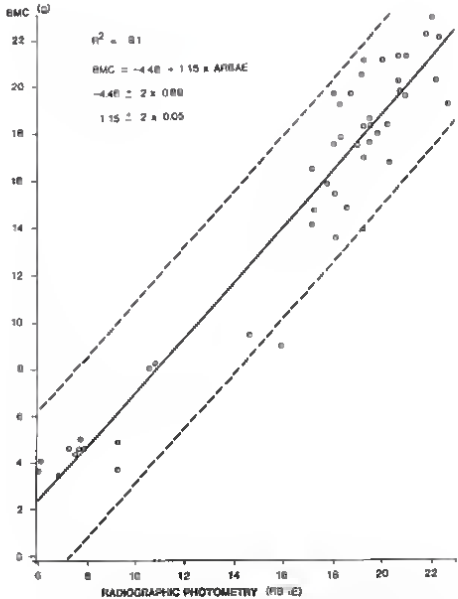

FIGURE 23— RELATIONSHIP BETWEEN BONE MINERAL CONTENT (BMC) OF 2 cm CROSS-SECTION TAKEN FROM EQUINE METACARPAL, 1 cm BELOW THE NUTRIENT FORAMEN, AND THE AVERAGE OF THE LATERAL PEAK, MEDIAL PEAK AND MIDPOINT, RADIOGRAPHIC BONE ALUMINUM EQUIVALENTS (RBAE) CORRESPONDING TO THAT CROSS-SECTION

TABLE 5. RADIOGRAPHIC PHOTOMETRY - CORRELATION COEFFICIENTS

	BMC	Breaking Load	Breaking Strength
Medial Peak*	.95	.95	.73
Center*	.95	.94	.73
Lateral Peak*	.91	.84	.65
Average*	.96	.93	.72

*Radiographic bone aluminum equivalents (RBAE) ($p<.001$).

sensitive non-invasive technique for determining BMC. One advantage of radiographic densitometry for the horse is that the entire McIII can be radiographed thus increasing the sample size. The exposure time of 1/15 sec made it ideal for use in the horse. Cortical area and bone geometry can be determined from radiographs. Cohn (1981) expected a standard error of the estimate for predicting ash weight by radiographic absorptiometry of ±6%. In vivo precision errors as low as 1% and as high as 15% have been reported. Photodensitometric BMC determinations of defleshed chicken bones had coefficients of variation from .88 to 1.22% these values are similar to those obtained by Meakim et al. (1981) for horses. Disadvantages include sources of error inherent in the use of X-ray. Such as those resulting from variation in energies of X-ray beams which cause nonuniformity in film exposure and radiation scattering. If uncontrolled, errors in BMC estimates may exceed 10%.

Photon Absorption. BMC estimated by photon absorption (figure 24) proved to be the most accurate predictor of BMC in horses (r = .991, p<.0001). The correlation between photon absorption and breaking strength was .66 (p<.001) (table 6). Photon absorption has been reported to be the most sensitive and accurate method for predicting BMC (Meyer et al., 1968; Wentworth et al., 1971, Smith et al., 1972; Sanchez et al., 1981). Precision errors were reported to be 2 to 4%. Smith et al. (1972) explained that the advantage of photon absorption over radiographic techniques was use of a monochromatic photon source as opposed to multiple energy levels of X-ray. Wentworth et al. (1971) adapted photon absorption for in vivo use in the horse. Although correlation coefficients for bone ash and photon absorption were high

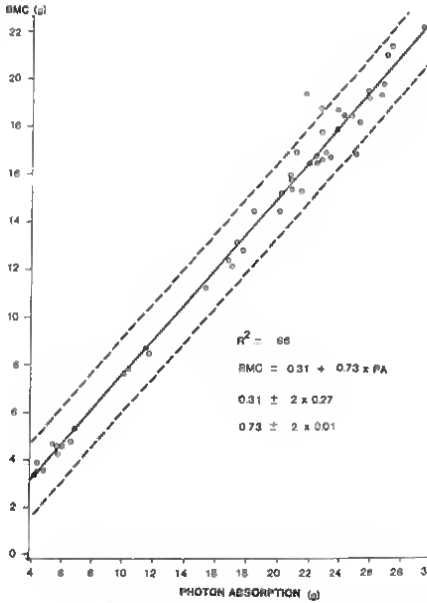

FIGURE 24 RELATIONSHIP BETWEEN BONE MINERAL CONTENT (BMC) OF 2 cm CROSS-SECTION DETERMINED BY ASHING AND PHOTON ABSORPTION ESTIMATED BONE MINERAL CONTENT

TABLE 6. PHOTON ABSORPTION AND ULTRASOUND CORRELATION COEFFICIENTS

	EMC	Breaking Load	Breaking Strength
Photon Absorption (g)	.99	.96	.66
Ultrasound (m/sec)	.92	.93	.82

p<.001

(.994) there were several disadvantages for its use in the equine. Because the animal's limb must be fixed in a jig, heavy tranquilization or anesthesia is required. The Mrill must be covered with a material which is similar to soft tissue in its absorption pattern. Scanning time is variable but averages 90 sec. The scan line is less that 1 cm and repositioning for follow up analysis may be difficult.

Ultrasound. The correlation of BMC with ultrasound velocity at the point of failure or mid point (figure 25) for all ages was .923 ($p<.0001$) (table 6). When ages were broken into groups, 1 d to 1.5 yr, 2 to 4 yr, 5 to 13 yr and 15 to 27 yr, the high correlations between BMC and ultrasound were only maintained for the 1 d to 1.5 yr group (.959, $P<.0001$). The correlation between ultrasound and breaking strength is represented in figure 26 ($r = .824$, $p<.0001$). The relationship between ultrasound and breaking strength is based on its association with bone density and the modulus of elasticity (Rich et al., 1966, Abendschien and Hyatt, 1972, Pratt, 1980, Greenfield et al., 1981). The correlation between ultrasound at the point of failure and the modulus of elasticity (figure 27) was .820 ($p<.0001$). In the data discussed above, ultrasound was calculated using the conventional "effective" velocity method of Pratt (1982). In order to better approximate the "path" ultrasound takes as it travels through the bone the velocity was determined using the formula $v = d/t$ with v = velocity, d = area (m^2) calculated by CAI, XAI, EAI and SCADS and t = transmission time.

Ultrasound velocities from bone sections from both 1 cm below the nutrient foramen and the point of failure were included in this analysis. Correlations were high between ultrasound and BMC when areas

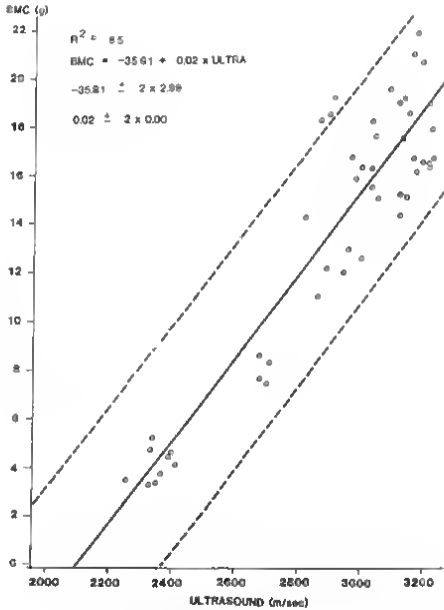

FIGURE 25. RELATIONSHIP BETWEEN BONE MINERAL CONTENT (BMC) OF 2 cm CROSS-SECTION DETERMINED BY ASHING AND ULTRASOUND VELOCITY.

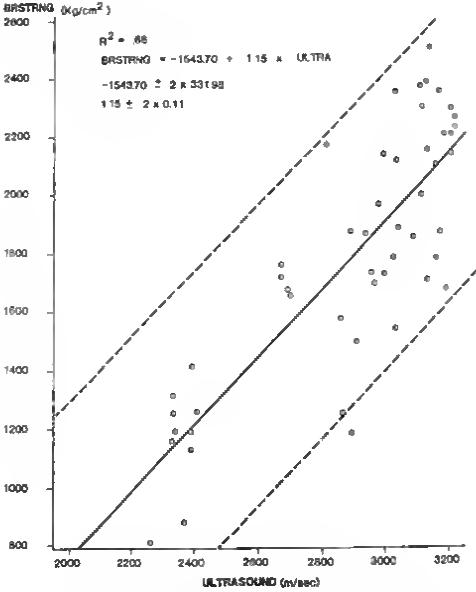

FIGURE 26 RELATIONSHIP BETWEEN BREAKING STRENGTH AND ULTRASOUND VELOCITY.

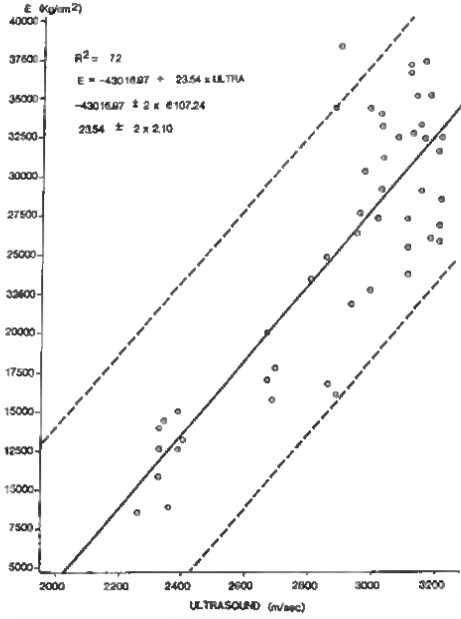

FIGURE 27. RELATIONSHIP BETWEEN MODULAS OF ELASTICITY AND ULTRASOUND VELOCITY.

were used to approximate d. The more accurate the estimate of area the higher the correlations between ultrasound and BMC (table 7) (figures 28, 29, 30, 31 and 32). As the correlation between BMC and ultrasound improved the correlation between breaking strength and ultrasound approached the relationship between actual BMC and breaking strength (table 7). Ultrasound estimates BMC better when expressed on an accurate area basis. Duncan's test of the means indicates all means are different (table 8). Rich et al. (1966) reported a correlation of .99 for mass of calcium per cm^2 and ultrasound transmission time. This suggests that ultrasound can accurately determine the chemical properties of bone.

Review of the literature suggest the most advantageous use of ultrasound may be in studies where animals are followed in a serial fashion and changes in velocity due to treatment can be detected (Rabia et al., 1983).

Although all techniques appear useful in predicting BMC in growing animals, further investigation of non-invasive techniques and their ability to predict BMC and breaking strength in mature animals is needed. It has been suggested and would appear to be confirmed by this study that a combination of photon absorption and/or radiographic photometry and ultrasound may be more useful in predicting the degree of mineralization and strength of bone.

TABLE 7. CORRELATION COEFFICIENTS FOR BMC AND BREAKING STRENGTH VS ULTRASOUND

	XRI/t[1]	CAI/t[2]	EAI/t[3]	SCADS/t[4]
BMC ($p<.0001$)	.892	.954	.954	.971
Breaking Strength ($p<.0001$)	.635	.741	.741	.663

[1]Radiographic area index/ultrasound time.

[2]Circular area index/ultrasound time.

[3]Elliptical area index/ultrasound time.

[4]Computer generated area index/ultrasound time.

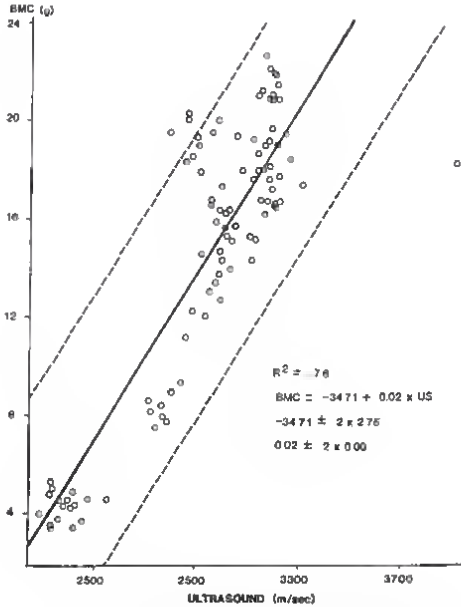

FIGURE 28. RELATIONSHIP BETWEEN BONE MINERAL CONTENT (BMC) OF 2 cm CROSS-SECTION AND ULTRASOUND VELOCITY CALCULATED USING THE OUTSIDE METACARPAL DIAMETER

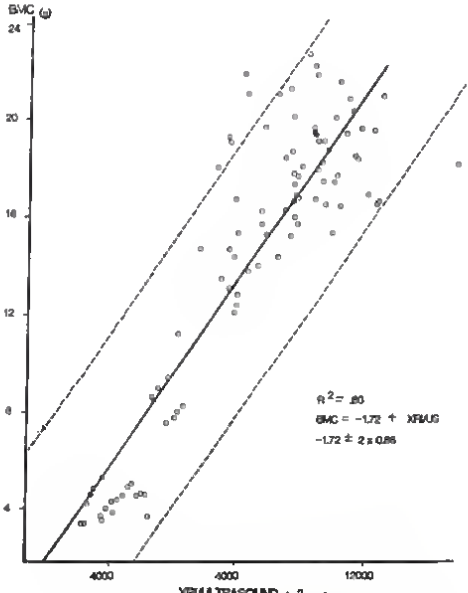

FIGURE 29. RELATIONSHIP BETWEEN BONE MINERAL CONTENT (BMC) OF 2 cm CROSS-SECTION AND ULTRASOUND VELOCITY CALCULATED USING CORTICAL AREA ESTIMATED FROM AN ANTERIO-POSTERIOR RADIOGRAPH OF EQUINE METACARPAL.

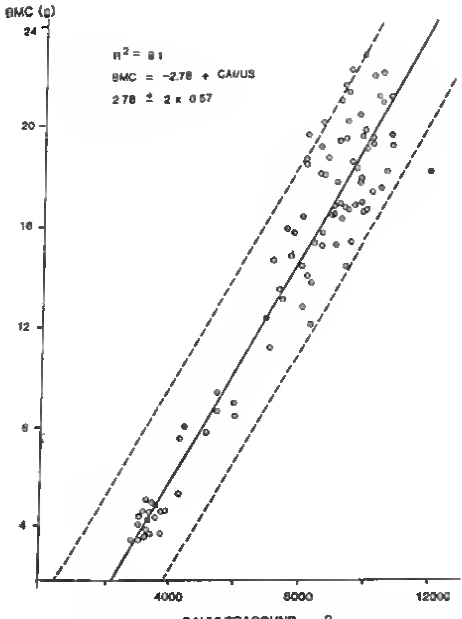

FIGURE 30. RELATIONSHIP BETWEEN BONE MINERAL CONTENT (%MC) OF 2 cm CROSS-SECTION AND ULTRASOUND VELOCITY CALCULATED USING CORTICAL AREA ESTIMATED FROM MEASUREMENTS TAKEN FROM BONE CROSS-SECTIONS.

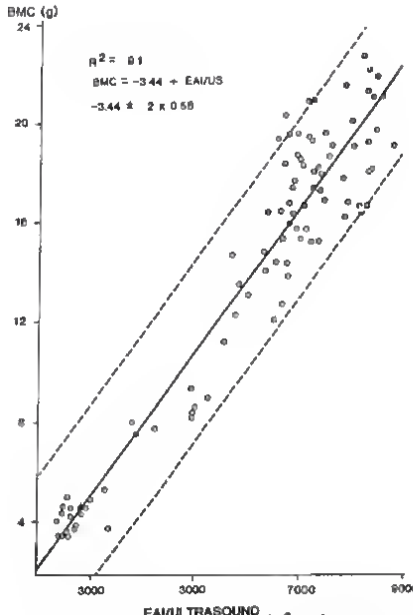

FIGURE 31. RELATIONSHIP BETWEEN BONE MINERAL CONTENT (BMC) OF 2 cm CROSS-SECTION AND ULTRASOUND VELOCITY CALCULATED USING CORTICAL AREA ESTIMATED FROM MEASUREMENTS TAKEN FROM BONE CROSS-SECTIONS

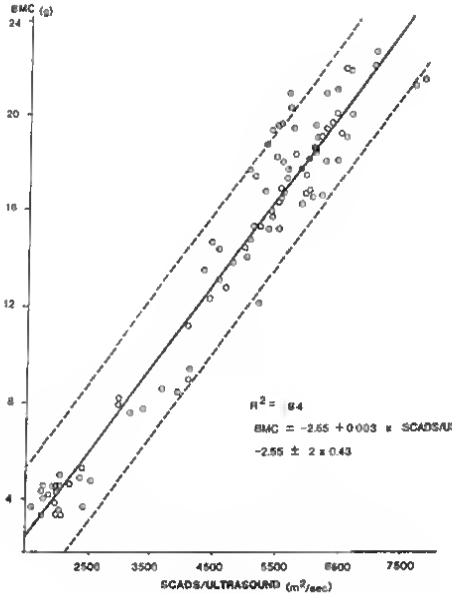

FIGURE 32. RELATIONSHIP BETWEEN BONE MINERAL CONTENT (BMC) OF 2 cm CROSS-SECTION AND ULTRASOUND VELOCITY CALCULATED USING CORTICAL AREA COMPUTED BY SCADS COMPUTER PROGRAM.

TABLE 8. MEANS FOR ULTRASOUND

Treatment	Means
XRI/t[1]	8460[a]
CAI/t[2]	7841[b]
EAI/t[3]	6179[c]
BD/t m/sec[5]	2931[e]

[1]Radiographic area index/ultrasound time

[2]Circular area index/ultrasound time

[3]Elliptical area index/ultrasound time

[4]Computer generated area/ultrasound time

a,b,c,d,e Values with different superscripts are different ($p < .01$)

CHAPTER IV
BONE DEVELOPMENT AND MINERAL INTERACTIONS

Iron Supplementation In Horses

Hematinics are widely and in many cases indiscriminately used in horses but there have been few studies to determine the value of various supplements and injectables reported in the literature. McGuire and Hansen (1968) in a review of the diagnosis of equine anemias stated that no evidence exist that iron or vitamin B_{12} deficiencies occur in the horse. Iron treatments used to improve packed cell volume (PCV) and hemoglobin (Hb) include ferrous sulfate (powered or liquid) and injectable iron preparations such as ferrous gluconate, iron citrate, iron adenylate and saccharated iron oxide. Other hematic drugs administered alone or in combination with iron preparations include thyroid hormone, ascorbic acid, folic acid, cobalt, B vitamins and copper (Kirkham et al., 1971).

Kirkham et al. (1971) conducted a study to determine the efficacy of some of the commonly used hematinics. They assigned 136 thoroughbreds in training to 12 treatment groups. The treatments consisted of oral and injectable iron preparations alone or in combination with other drugs. Blood samples were obtained at 2, 4 and 8 wk intervals. Only one treatment out of 12 showed improvement in PCV and Hb ($P<.05$ and $P<.01$). No iron supplement or injection alone improved Hb or PCV. Two other important factors also decrease the chance of iron deficiency in horses. First, typical diets fed to horses

generally contain more than adequate amounts of iron. Speir et al (1977) reported levels of 750 ppm iron in an oat-barley and alfalfa hay diet. Secondly, horses have the abilities to store large reserves of RBC in the spleen that can be used to increase the RBC count by 50% during periods of stress (Thomas and Fregin, 1981).

Iron deficiency anemia has been reported in cases of acute blood loss (Smith et al., 1984). Smith et al. (1984) reports that if blood loss results in lowering the PCV by 50%, the horse may have lost as much iron as is stored in the liver and spleen.

Iron Absorption

Motty (1971) stated iron absorption takes place in two phases with mucosal uptake in the small intestine directly related to concentration of iron. Transfer to plasma is slower than mucosal uptake and is dependent on ATP and oxidative metabolism. The small intestine is the site of iron absorption. Iron enters the plasma in the ferrous state and is changed to ferric iron. The B_1 globulin, transferrin then binds with the ferric iron and can be transported via the blood (NRC, 1980). The mechanism for the control of iron absorption is important to the metabolism of iron because the amount of iron in the body is regulated by absorption (Underwood, 1971). Motty (1971) proposed one theory of the control of iron absorption based on an acceptor protein, apoferritin, contained in the epithelial cell apoferritin takes up iron until it is saturated and then no more iron could be passed through the cell into the body.

Another theory of iron absorption is based on development of epithelial cells in the intestinal villae. Mucosal cells take up iron

at the time of formation in amounts dependent on body stores. If an iron deficiency exists, cells of low iron content, which can absorb more iron are formed. If an iron overload exists, iron may enter the cell but it cannot be passed on and is lost by desquamation of the cell (Murry, 1971). Other theories on the control of iron absorption include a humoral factor associated with erythropoesis and transferrin saturation. There appears to be multiple factors involved in the control of iron absorption because no one mechanism can explain all aspects of absorption.

Iron Metabolism

Iron was among the first nutrients to be recognized as a treatment for a condition now known as iron-deficiency anemia (NRC, 1980). The essentiality of iron is associated with its function in respiration and oxygen transport. Hemoglobin, myoglobin, the cytochromes and other enzymes contain iron.

Much of the early work with iron was done to identify its relationship with hemoglobin formation. The RBC (hemoglobin) contains the majority of iron (3 to 4 g) in the body. Plasma iron is also found coupled to the iron transport protein, transferrin which can carry two molecules of iron per transferrin molecule. Bone marrow contains significant quantities of iron, with transferrin molecules transporting iron to RBC precursors. The marrow also functions in the reticuloendothelial (RE) system as a site for iron transport, storage and RBC destruction. The RE cells are responsible for destruction of senescent RBC, digestion and redistribution of iron. The RE system includes the spleen, liver, bone marrow and lymph nodes. It is the role

of the liver in the RE system that makes it important as a storage depot of iron (Rigby, 1971).

Iron Storage

Iron metabolism studies are complicated because of internal recycling. Iron from destroyed RBC is preferentially passed to plasma transferrin by reticuloendothelial cells. It is then carried to the marrow for RBC formation (Thomas, 1970).

A second factor that complicates metabolism studies is that body stores of iron influence absorption (Mucosal Block Theory). About 25% of body iron is in the storage forms, ferritin and hemosiderin, found in liver, spleen, bone marrow and other tissues.

Iron Toxicity

Campbell (1961) described the acute toxicity of iron in pigs varying in age from 3 to 10 d. The pigs were given 0.6 mg Fe ($FeSO_4$)/kg body weight by stomach tube. The animals vomited 20 to 30 minutes after dosing and scouring occurred shortly afterwards. Within one hour animals showed signs of shivering and hyperpnoea, with staggering, incoordination and muscular tremors. Some of the pigs developed posterior paralysis and tetanic convulsions. Campbell et al. (1961) also reported plasma iron values increased from 125 g/100 l at 2 d of age to 401 g/100 l at 5 d of age after that they decreased to 55 g/100 l by 10 d.

In a study conducted by Ammerman et al. (1967) with six calves fed an iron depletion diet, greater iron absorption was noted when body stores were reduced. Following dosing with Fe^{59} the liver accumulated the highest concentration of Fe^{59} followed by the spleen. Experiments

with sheep indicated the concentration of Fe^{59} in the spleen approached or exceeded that in the liver in non iron depleted sheep

In an attempt to determine the biochemical basis of toxicity or iron over load Witzleben and Choffey (1966) injected rabbits with iron sulfate. The authors observed disturbances in hepatocellular oxidative enzyme activity and suggested this may be a probable biochemical basis for iron toxicity. Buck et al. (1976) stated that when the binding capacity of transferrin is exceeded serum iron concentrations rise rapidly. When the serum passes through the liver and spleen much of the iron is removed and may result in organ damage.

Iron Interactions

The effects of excess dietary iron must be examined not only in terms of iron overload and toxicity but also in terms of antagonistic interactions among other minerals. The elements most often cited as having antagonistic relationships with iron are phosphorus, copper, zinc and manganese.

Thomas (1970) stated that the similar chemistry of the transition metals (Zn, Fe, Mn and Cu) allows such cations to be substituted for one another in many enzyme systems where these minerals act as chelating agents, but not when they act in electron transfer.

Iron-Phosphorus

Koong et al. (1970) demonstrated that calves fed supplemental iron as iron citrate at 500, 1000 and 2000 ppm had increased serum iron and lowered serum phosphorus. Based on their studies, they concluded that calves could tolerate 2000 ppm. Early studies also reported adverse effects of high iron intake on serum phosphorus levels. Cox et al.

(1931) reported that when ferric salts were fed to guinea pigs at levels greater than phosphorus the ash, calcium and phosphorus of bones was reduced. The decreased bone mineral was associated with decreased serum phosphorus.

Brock and Diamond (1934) found that the dialysis of ferric ammonium citrate across cellophane membranes was decreased by the addition of sodium phosphates to the iron solution. They suggested that a similar condition may occur in the intestine and that by forming an insoluble compound iron and phosphorus might hinder the absorption of each mineral. Based on this supposition the authors designed an experiment to determine if addition of iron to a nontechitogenic diet could reduce the absorption of phosphorus to the extent rickets could be produced. Weanling rats were fed the following diets; a control, the Steenbock rachitogenic, a ferric chloride, ammonium chloride, ferric chloride and added phosphorus, ferric ammonium citrate, ferrous chloride, reduced iron and an organic iron diet. The results indicated that high dietary iron decreased serum phosphorus and produced histological evidence of rickets. Brock and Diamond (1934) also found that the effects of excess iron could be overcome by increasing the phosphorus level of the diet. Rehm and Wisteen (1940) added 2 levels of supplemental ferric chloride to the casein-corn starch diets of experimental rats. Intake of control diets was equalized with decreased intake of the high iron diets so all rats consumed the same amount. The rats were killed after 4 weeks both total bone ash and total body phosphorus were decreased 20 to 28%.

O'Donovan et al. (1963) allotted 24 pigs to four groups and fed a control diet containing 15 ppm iron or added 80, 400 and 2000 ppm iron

as ferrous sulfate. They determined that 15 ppm iron decreased performance and 80 ppm increased, average daily gain, hemoglobin and PCV. Pigs receiving 2000 ppm iron or less performed well. However, O'Donovan et al. (1963) observed rickets with decreased serum phosphorus and percent femur ash when pigs were fed 5000 ppm iron at 2500 ppm iron if the phosphorus level was lowered from .63 to .35%.

Furugouri (1972) reported results similar to O'Donovan et al. (1963) when they fed 60 to 70 d old pigs diets supplemented with 202, 1102, 3102, 5102 and 7102 ppm iron as ferrous sulfate. Serum phosphorus was linearly reduced with increasing iron and length of time on feed. Femur ash, phosphorus and calcium decreased linearly in pigs fed 5102 ppm and 7102 ppm iron. Furugouri (1972) states that extra dietary iron produced phosphorus deficiency signs in young growing pigs.

Iron-Copper-Zinc-Manganese

The iron-phosphorus interaction takes place within the lumen of the intestine and involves the precipitation of an Fe-P mineral complex. The antagonism between iron-copper-zinc provides examples of other important types of interactions. Mineral interactions may occur during the process of absorption in the form of competition for binding sites or transport compounds, mineral interactions may interfere with enzymatic functions by substitution, there may be competition at the target organ or cell and interactions may involve metabolic disturbances that lead to excessive secretion and/or accumulation of elements (Davis, 1980).

Cotzias (1958) fed high levels of iron to rats and noted decreased absorption of manganese and iron. The interaction of manganese with iron appears to occur in the lumen of the intestine with precipitation of an iron-manganese complex (Thomas, 1970).

Kaincki et al (1967) reported that diets supplemented to contain 661 ppm iron as ferrous sulfate decreased copper levels in the liver and spleen of young growing pigs. The authors stated that high dietary iron could increase the copper requirement and concentration at active sites of iron metabolism, other than the liver. They concluded that the iron to copper ratio affected either absorption or transportation to metabolic sites. McNaughton and Day (1979) found that a ratio of iron to copper of 5:1 maximized weight gains of chicken while 10:1 maximized Hb and PCV but decreased gains and 20:1 adversely affect gains, Hb and PCV.

Humphries et al (1985) states that 350 ppm iron can deplete liver copper reserves in calves and 800 ppm iron can cause a copper deficiency. Standish et al. (1969) fed 24 calves for 84 days diets containing 0, 400, 1600 and 2400 ppm supplemented iron as ferrous sulfate. The authors reported that plasma copper was depressed by 1600 ppm iron. Standish et al. (1969) also reported that as iron in the liver increased zinc and copper decreased. Standish et al (1971) fed two levels of iron (100 and 1000 ppm) to steers. The authors reported decreased average daily gain in steers fed high iron. The high iron diet caused tissue iron accumulation. High iron diets also lowered copper and zinc levels in liver and increased zinc in the spleen.

McGhee et al. (1965) evaluated the effects of various levels of iron and copper on growth, gain and survival in poultry. Two groups of birds were used. In group 1, 5 ppm copper was added to the basal diet while iron level was varied from 50 ppm to 1600 ppm. Group 2 had iron added at 40 ppm and the copper level was varied from 5 to 160 ppm. The

highest weights occurred in group 1 when iron was added at 50 ppm and copper 5 ppm. Weights decreased with increasing levels of iron and at 200 ppm iron 10% mortality occurred. In group 2 as much as 40 ppm copper was tolerated by the birds with no detrimental effects However, decreased weight gain and increased mortality was observed at 80 ppm or 160 ppm copper with 40 ppm iron.

Early work on the iron copper relationship suggests that iron transport is dependent on a copper bound protein carrier (ceruloplasmin) for movement from depository tissues (Sourkes et al., 1968). This interaction provided the link between iron and copper in anemia. There is also evidence that copper is preferentially bound to transferrin in the small intestine when copper and iron levels are high (El Shobaki and Kunnel, 1979). Zinc may also compete for binding sites on transferrin and the copper transport protein metallothionein. It appears that the interactions among zinc, copper and iron and their transport mechanisms may account for many of the antagonistic problems seen when ratios of these elements are varied from the ratios of requirement levels (Davie, 1980).

Influence of Dietary Iron on Growth, Tissue Mineral Composition, Apparent Phosphorus Absorption and Chemical and Mechanical Properties of Bone in Ponies

Although no evidence exists that iron deficiencies occur in horses iron containing hematinics are used widely and in many cases indiscriminately by members of the horse industry. Dietary iron supplements and injectables are used to improve packed cell volume (PCV) and hemoglobin (Hb). While there is little evidence of the efficacy of hematinics, there may be a potential for iron toxicity. There may also

be potential for antagonistic interactions between phosphorus and iron and the trace minerals, copper, zinc and manganese. The importance of phosphorus, copper, zinc and manganese to bone development is well established (Underwood, 1971).

Excess iron in the diets of monogastrics has been reported to reduce serum phosphorus and decrease bone mineralization (Brock and Diamond, 1934, O'Donovan et al., 1963, Parnangnl, 1972). Humphries et al. (1985) reported that 350 mg/kg dietary iron can deplete liver copper reserves in calves and 800 mg/kg iron can produce signs of copper deficiency. Standish et al. (1969) reported 1600 mg/kg iron depressed plasma copper levels and lowered liver copper and zinc. Cotzias (1958) reported the absorption of manganese is decreased in rats when high levels of iron are fed.

Recent suggestions by Bridges et al. (1984) and Knight et al. (1985) that problems in equine bone development such as osteochondrosis are caused by nutrition, specifically copper deficiency and perhaps zinc and molybdenum makes information on trace mineral interactions valuable. The objectives of this study were to determine if high dietary levels of iron either alone or by mineral interactions would cause iron toxicity and/or affect growth, mineral composition of selected tissues, apparent phosphorus absorption and the chemical or physical properties of third metacarpals of ponies.

Experimental Procedure

Six weanling and six yearling ponies were alloted to three groups and fed 0, 500 or 1000 mg/kg supplemental iron for 90 d (table 9). The supplemental iron was added to the basal diet (table 10) as ferric

TABLE 9 EXPERIMENTAL DESIGN

Diets	1	2	3
Basal	+	+	+
Ferric citrate			
Fe, mg/kg	–	500	1000
Yearlings[a]	2	2	2
Weanlings[b]	2	2	2

[a]All yearlings were colts.

[b]One weanling filly was assigned each to diet 1 and diet 3. All other weanlings were colts.

TABLE 10. COMPOSITION OF BASAL DIET[1]

Ingredient	Percent
Coastal bermuda hay, pelleted, ground	35.00
Corn, ground	40.50
Soybean meal (44% CP)	17.50
Alfalfa, dehy.	5.00
Bio. Fos.	0.25
Ground limestone	0.25
Vit premix[2]	0.25
TM premix[3]	0.25
Salt	0.50
Mono. propionate	0.50

[1]Experimental diets formulated by adding ferric citrate (15.8% iron) to the diet at the expense of corn.

[2]Premix provided 11,000 USP units Vit. A 580 USP units Vit B_3 and 11 IU Vit. E/kg of the diet.

[3]Premix provided 10.8 mg manganese, 40.0 mg iron, 7.5 mg copper, 39.6 mg zinc, 0.2 mg cobalt and 0.2 mg iodine/kg of the diet.

citrate. The analysed composition of the experimental diets is reported in table 11.

Ponies were dewormed before the trial started and fecal samples were collected periodically to insure the animals were not stressed by parasites. Initial weights were recorded along with baseline blood values at the start of the trial. Weights were recorded and blood samples were collected every 3 wk throughout the trial. Serum iron and iron binding capacities were determined spectrophotometrically using a commercial kit (Ferro-Chek II, Hyland Diagnostics, Deerfield, Illinois). Red blood cell counts, white blood cell counts, packed cell volume and hemoglobin were determined using an electronic blood cell counting system (ZBI-6 System, Coulter Electronics, Hialeah, Florida). Serum was separated and frozen for later analyses for calcium, phosphorus, copper, zinc and manganese. Phosphorus was determined by the method of Fiske and Subbarow (1925). Serum calcium, copper, zinc and manganese were determined by atomic absorption spectrophotometry as described by Sunderman and Carroll (1965). On d 73 the six yearling ponies were placed in digestion crates. After 2 d of adaptation total urine and feces were collected twice daily and samples pooled and sub-sampled for analysis of calcium and phosphorus. At the end of 90 d on trial all yearlings and one weanling from each group were euthanized. Liver, kidney, heart and the left and right third metacarpals (McIII) were collected. Tissue calcium, manganese iron, copper, zinc and magnesium were determined by atomic absorption spectrophotometry according to the methods recommended by the manufacturer (Analyzeue, 1964). The right and left McIII were

TABLE 11. ANALYSED COMPOSITION OF EXPERIMENTAL DIETS

Diets	1	2	3
Analyses, DMB			
CP%	18.37	17.99	17.70
Ca%	0.75	0.92	1.14
P%	0.45	0.47	0.47
Mg%	0.19	0.19	0.19
Na%	0.18	0.30	0.33
K%	1.27	1.30	1.21
Fe mg/kg	436.00	893.00	1440.00
Cu mg/kg	11.00	8.06	10.37
Zn mg/kg	71.00	53.90	53.43
Mn mg/kg	42.64	41.60	40.25
Mo mg/kg	1.15	1.21	1.50
Se mg/kg	0.14	0.17	0.18

collected. Anterior-posterior (AP) radiographs of MrIII were taken with an aluminum stepwedge exposed simultaneously as a reference standard. A photometric scan of the stepwedge and bone (1 cm below the nutrient foramen) provided an estimate in radiographic bone aluminum equivalents (RBAE) from which BMC was estimated by the regression equation BMC = -4.46 + 1.15 RBAE. After radiographs were taken all tissues were removed and MrIII were wrapped in .9% saline soaked gauze, placed in plastic bags and frozen at -15°C until further analysis.

High frequency ultrasound was used to evaluate MrIII by measuring time elapsed as sound traveled from one transducer placed on the lateral side of MrIII and a second transducer placed on the medial side, at the midpoint of the bone. The time required for sound to travel through the bone was divided by the bone diameter. Velocity is reported in m/s. The ultrasonic generator was a Panametrics 5055 transmitter/receiver which was coupled to KB Aerotech 1MHz transducers[1]. The transducer signal was displayed on a Hewlett Packard oscilloscope[2]. Calipers were used to measure lateromedial diameters of bone.

A Lunar Radiation Corporation analyzer[3] was used for dual photon absorptiometry which included a collimated radionuclide source emitting a radiation beam at two separate energies. Metacarpals were submerged in a 0.9% saline solution which has a similar absorption pattern to soft tissues. The scan line is 0.35 cm in width, six lines were scanned at the midpoint and BMC is reported in grams per 2.1 cm section.

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[1]Panametrics, Waltham, MA.

[2]Valleytrine Lab Incorporated, Model 1023AX, Kenston, NJ.

[3]Lunar Radiation Corporation, Model DP_3, Madison, WI.

Wet femurs were tested by three point bending on an testing machine[1] at a deformation rate of 50 mm/min. Broken bones were reassembled and 2 cm cross-sections were removed at the point of break and 1 cm below the nutrient foramen. These cross-sections were photographed and digitized using a microcomputer with a graphics tablet, this data was then transferred to a main frame computer program (SCADS) which computes the vertical area and the moment of inertia without assuming geometric shape (Piotrowski and Kellman, 1973). Caliper cross-sectional measurements from bones and radiographs were made and designated as follows: AP bone diameter (B), AP medullary diameter (b), lateromedial (LM) bone diameter (D), and LM medullary diameter (d). Equations for calculating area indices were, circular cross-sectional area index (CAI) (B^2-b^2), and elliptical cross-sectional area index (EAI) (BD-bd). The bone sections were ashed after extracted then ashed at 600 C for 12 hr. Bone ash was expressed as grams per 2 cm section and defined as BMC.

Results and Discussion

Animal Growth. Supplemental iron fed at 500 and 1000 mg/kg had no effect on feed intake, average daily gain or feed per unit gain (table 12). Depressed feed intake and average daily gain has been expected in steers fed 400 mg/kg iron (Standish et al., 1969). Reduced weight gains in steers fed excess dietary iron has also been reported by Coup and Campbell (1964), Kenny et al, (1970) and Standish et al. (1971). Similar results have been reported by Standish and Ammerman (1971) for lambs fed 1600 mg/kg supplemental iron.

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[1]Instron Corporation- Canton, MA.

TABLE 12. EFFECT OF DIETARY IRON ON INTAKE AND WEIGHT GAIN

Supplemental Iron mg/kg	Days On Feed	Average Daily Feed Intake, kg	Average Daily Gain, kg	Feed Per Unit Gain	Initial[1] Weight, kg	Final[1] Weight, kg
0	90	5.28[a]	0.48[a]	8.91[a]	204.35[a] ± 49	231.25[a] ± 50
500	90	6.10[a]	0.55[a]	14.48[a]	238.53[a] ± 92	282.25[a] ± 110
1000	90	5.88[a]	0.52[a]	14.73[a]	234.00[a] ± 38	281.00[a] ± 24
Period Means						
1		5.39[a]	0.56[a]	10.74[a]		
2		5.67[a]	0.61[a]	8.79[a]		
3		5.66[a]	0.33[a]	13.06[a]		
4		6.25[a]	0.54[a]	17.89[a]		

a,b,c Values in same column with different superscripts are different ($p<.05$)

[1] Body weight ± SE.

O'Donovan et al. (1963) and Furugouri (1972) reported that pigs receiving less than 3000 mg/kg iron performed well but when iron levels were increased to 4000 to 5000 mg/kg, average daily gain and feed intake was reduced ($P<.05$). Although pigs seem to tolerate high iron diets other monogastrics including chickens and turkeys have demonstrated reduced growth at 400 or 500 mg/kg iron (Waerpel and Halloua, 1961, McGhee et al., 1965). The growth performance of ponies appears to be unaffected by supplemental iron at 500 mg/kg and 1000 mg/kg.

Blood and Serum Composition. The mean blood values associated with iron status in animals are given in table 13. Iron supplementation did not increase the number of RBC, Hb, PCV or serum iron. There is some indication iron was being accumulated at the 1000 mg/kg level. Unbound iron binding capacity was lowered in the high iron group. The percent iron saturation of serum was also increased for the 1000 mg/kg supplemented group

Period effects were observed with serum iron increasing ($P<.05$) from 32.42 g/dl to 178.33 g/dl in the first 28 d. Period effects were also observed for serum iron and total iron binding capacity.

There were not treatment effects (table 14) on serum P although serum P was decreased ($P<.05$) at 56 d. No effect was observed on serum calcium, manganese or copper. Serum zinc, however, was lowered ($P<.05$) by iron supplementation at the 500 mg/kg iron level. Period effects were also noted for serum zinc.

The ability of iron supplementation to increase RBC, PCV, Hb is influenced by age, species, iron status, dietary mineral interactions and other factors. Harvey et al. (1984) reported PCV and Hb decreased

TABLE 13 EFFECTS OF DIETARY IRON ON CERTAIN HEMATOLOGICAL VALUES IN PONIES

Supplemental Iron mg/kg	RBC x 10 8/liter	WBC x 10 3/liter	HB g/dl	PVC liters/liter	PP g/liter	CT MS	Serum Iron ug/dl	UIBC ug/liter	TIBC ug/liter	% Saturate Iron
0	8.39[a]	8.83[a]	12.09[a]	33.01[a]	3.03[a]	11.44[a]	135.38[a]	384.06[a]	639.81[a]	35.30[ab]
500	8.8[a]	9.82[a]	12.92[a]	33.31[a]	7.03[a]	11.33[a]	133.33[a]	383.41[a]	633.61[a]	36.38[a]
1000	8.4[a]	9.33[a]	11.99[a]	33.13[a]	6.03[a]	11.27[a]	163.83[a]	231.63[a]	393.46[a]	41.62[a]
Period Means										
Initial	8.7[a]	10.60[a]	11.36[a]	33.31[a]	3.11[a]	10.33[a]	132.63[a]	233.32[a]	339.94[b]	35.66[a]
1	8.9[a]	8.81[a]	11.33[a]	33.92[a]	8.03[a]	11.33[abc]	138.33[a]	286.33[a]	463.01[ab]	38.03[ab]
3	8.6[a]	9.83[a]	11.81[a]	33.14[a]	6.37[a]	11.35[a]	136.94[ab]	364.36[a]	421.50[a]	33.30[a]
3	8.3[a]	9.98[a]	12.24[a]	33.56[a]	6.88[a]	11.03[bc]	169.31[ab]	378.38[a]	448.30[a]	38.43[a]
4	9.1[a]	9.29[a]	12.37[a]	33.63[a]	5.93[a]	11.68[ab]	140.13[ab]	368.82[a]	410.94[ab]	36.60[a]

[a,b,c]Values in same column with different superscripts are different ($p<.05$).

RBC = red blood cell, WBC = white blood cell; HB = hemoglobin, PVC = packed cell volume; PP = proteins, CT = clotting time, UIBC = unsaturated iron binding capacity; TIBC = total iron binding cell capacity.

TABLE 14. EFFECT OF DIETARY IRON ON SERUM COMPOSITION OF PONIES

Supplemental Iron mg/kg	Serum Minerals mg/kg				
	Calcium	Phosphorus	Manganese	Copper	Zinc
0	12.26[a]	5.25[a]	5.63[a]	1.53[a]	0.75[a]
500	12.14[a]	5.23[a]	9.14[a]	1.50[a]	0.56[b]
1000	12.00[a]	5.61[a]	9.00[a]	1.60[a]	0.74[a]
Paired Means					
Initial	12.11[a]	6.07[a]	9.74[a]	1.59[a]	0.63[b]
1	11.66[a]	5.45[ab]	4.89[a]	1.68[a]	0.61[b]
2	12.62[a]	4.65[b]	8.16[a]	1.78[a]	0.91[a]
3	12.11[a]	5.09[ab]	7.03[a]	1.24[a]	0.56[b]
4	12.05[a]	5.51[ab]	10.07[a]	1.36[a]	0.66[b]

a,b,c Values in same column with different superscripts are different ($p<.05$).

significantly during the first two weeks in newborn foals and stayed in the lower portion of the normal adult horse range throughout the first year of life. They reported a PCV for weanlings .34 liters/liter and .36 liters/liter for yearlings. Hemoglobin values of weanlings and yearlings averaged 12.2 g/dl. Red blood cell counts of weanlings and yearlings were 9.5 x 10^{12}/liter.

Standish et al (1969) reported no change in PCV and Hb in 217 to 253 kg steers supplemented with increasing levels of iron. Koenig et al (1970) reported PCV and Hb were increased by iron supplementation in younger calves.

O Donovan et al. (1963) placed early weaned (8.21 lb) pigs on trial and noted an increase in Hb or PCV by supplementation with 400 or 2000 mg/kg iron. Farugouri (1972) noted increases in Hb in pigs when the diet was supplemented with 1102, 5102 and 7102 mg/kg iron but noted an increase when 3102 mg/kg iron was added.

Kirkham et al. (1971) assigned 136 thoroughbreds in training to 12 treatment groups. Each group received an iron supplement alone or in combination with other drugs with hematinic activity. Only one treatment out of 12 improved PCV and Hb ($P<.05$ and $P<.01$). No iron supplement or injection alone improved PCV or Hb.

There is little evidence of iron deficiency anemia in horses (McGaire and Hesson, 1968). Typical diets fed to horses generally contain more than adequate amounts of iron (Speis et al., 1977). Another important factor is the ability of horses to store large reserves of RBC in the spleen that can be used to increase the RBC count by 50% during periods of stress (Thomas and Fregin, 1981).

Brock and Diamond (1934) fed diets high in iron to rats and noted reduced serum phosphorus and histological evidence of rickets. Cox et al. (1931) in another early study reported that when guinea pigs were fed iron at greater levels than phosphorus, bone mineral was decreased with an associated decreased serum phosphorus.

Kemmo et al. (1970) noted increasing iron supplementation (500, 1000, 2000 mg/kg) increased serum iron and decreased serum phosphorus. Furugouri (1972) reported that serum phosphorus decreased linearly with increasing iron and increasing length of time on feed in pigs fed 202, 1102, 3102, 5102 and 7102 mg/kg iron.

There was little evidence that iron supplemented at 500 or 1000 mg/kg had adverse effects on serum minerals in this study. The only interaction observed between iron and zinc was at the 500 mg/kg level.

McGuire et al. (1985) noted decreased serum zinc in calves fed 1000 mg/kg supplemental iron ($FeSO_4$). Associated with lowered zinc in serum, McGuire et al. (1985) also reported lowered spleen and pancreas zinc.

Tissue Mineral Composition. The mean values for the mineral composition of the various tissues are shown in table 15. The iron content of the liver of the high iron group was higher (721.97 mg/kg, $P<.05$) than the control group (437.87 mg/kg) and the 500 mg/kg group (496.80 mg/kg). Iron did not accumulate in the spleen or kidney. There was no difference in liver and kidney phosphorus between groups but the 500 mg/kg group had lowered phosphorus in the spleen. Copper and manganese in the spleen were lowered in the 1000 ppm iron supplemented group.

TABLE 15. EFFECT OF DIETARY IRON ON THE TISSUE MINERAL COMPOSITION OF PONIES

Tissue	Supplemental Iron, mg/kg	Tissue mineral, mg/kg DMB					
		Iron	Phosphorus	Copper	Zinc	Manganese	Calcium
Liver	0	437.87[b]	0.92[a]	16.29[a]	165.28[a]	7.38[a]	295.70[a]
	500	496.80[b]	0.95[a]	16.54[a]	129.97[b]	7.09[a]	308.30[a]
	1000	721.07[a]	0.89[a]	15.66[a]	132.21[b]	6.68[a]	370.50[a]
Spleen	0	2.65[a]	1.45[a]	11.13[a]	126.13[a]	1.53[a]	282.93[a]
	500	4.17[a]	1.26[b]	8.26[ab]	113.05[a]	1.31[ab]	240.47[a]
	1000	5.12[a]	1.39[ab]	7.81[b]	124.96[a]	0.87[b]	254.29[a]
Kidney	0	183.67[a]	1.17[a]	24.75[a]	147.79[a]	4.97[a]	6082[a]
	500	182.23[a]	1.26[a]	28.51[a]	144.79[a]	5.39[a]	705[a]
	1000	252.43[a]	1.32[a]	23.52[a]	130.84[a]	5.51[a]	692[a]

[a,b,c]Values in same column with different superscripts are different ($p<.05$).

Liver zinc content was lower in both iron supplemented groups (table 15). Spleen and kidney zinc levels were unchanged by iron supplementation. Tissue calcium levels were not affected by any treatment. Iron supplementation did not affect any of the minerals analyzed in the kidney.

Ammerman et al. (1967) reported Fe^{59} accumulation in the liver of iron depleted calves. However, in non-iron depleted sheep the authors reported Fe^{59} accumulation in the spleen approached or exceeded that in the liver. Furugouri et al. (1972) observed a linear increase in liver iron stores with increasing iron supplementation in pigs. The authors suggest that the mucosal block theory mechanism can be only partially applied when excessive iron supplementation occurs.

Buck et al (1976) stated that when high levels of iron are absorbed and the binding capacity of transferrin is exceeded serum iron concentrations rise. When the serum passes through the liver and spleen excess iron is removed and stored in these organs.

Kainoki et al. (1967) reported that diets containing 661 mg/kg iron ($FeSO_4$) decreased copper levels in the liver and spleen of young pigs. The authors suggested the dietary iron to copper ratio affected either copper absorption or transportation to metabolic sites. McNaughton and Day (1979) found that a ratio of 20 1 (Fe Ca) adversely affected gains, PCV and Hb in growing chicks.

Humphries et al. (1985) reported 350 mg/kg iron depleted copper reserves in calves and 800 mg/kg produced copper deficiency signs. Standish et al. (1969) and Standish et al. (1971) found increased dietary iron lowered copper and zinc levels in the liver.

Cotzias (1958) fed high levels of iron to rats and noted decreased absorption of manganese and iron. The interaction of manganese may occur in the intestine with a precipitation of an iron-manganese complex (Thomas, 1970)

El Shabaki and Rumsel (1979) reported that copper may be preferentially bound to transferrin in the small intestine when copper and iron levels are high. Zinc may also compete for binding sites on transferrin and the copper transport protein metallothionein. It appears that the interactions among zinc, copper and iron and their transport mechanisms may account for many of the antagonistic reactions seen when ratios of these elements are varied from the ratios of requirement levels (Davis, 1980).

Apparent Absorption of Calcium and Phosphorus The average coefficients for the apparent absorption of phosphorus and calcium are shown in table 16. The apparent absorption of phosphorus and calcium was not affected by dietary iron supplementation. The average apparent absorption of 35.63% for phosphorus and 50.9% for calcium agrees with values reported by Schryver et al. (1971) and Schryver et al. (1970).

Bone Growth and Development. Bone mineral content at the point of failure and breaking load increased ($P<.05$) in the iron supplemented groups (table 17). Differences ($P<.05$) in BMC were detected by photon absorption. No differences were noted in breaking strength and elastic modulus. Ultrasound evaluation did not show differences in the velocity of sound through the McIII. The high iron group had a greater ($P<.05$) cortical area as determined by SCANS, circular area index and elliptical area index.

TABLE 16 EFFECT OF DIETARY IRON ON APPARENT ABSORPTION OF CALCIUM AND PHOSPHORUS[1,2]

Supplemental Iron mg/kg	Phosphorus	Calcium
0	38.6[a]	48.9[a]
500	32.5[a]	50.7[a]
1000	35.7[a]	52.8[a]

[1]Data collected during day 75-80

[2]Data reported as percent apparent absorbed mineral.

[a,b,c]Values in same column with different superscripts are different ($p<.05$).

TABLE 17. EFFECT OF DIETARY IRON ON PROPERTIES OF THIRD METACARPALS OF PONIES

Supplemental Iron mg/kg	Bone Mineral Content g	Breaking Load kg	Breaking Strength kg/cm^2	Elastic Modulus kg/cm^2	Area BCA/B cm^2	Circular Area Index cm^2	Elliptical Area Index cm^2	Photon Absorption g	Ultrasound m/s
0	5.62^a	878 62^c	2543^a	3867^a	2.41^b	4.38^b	3.02^b	7.28^c	2920^a
500	6.18^b	865 33^b	2838^b	38562^a	2.57^a	4.38^b	3.23^a	7.68^b	2822^a
1000	6.84^a	824 50^a	2842^a	38661^a	2.46^a	5.14^a	3.87^a	8.17^a	2846^a

[1]Measurements determined on 2 cm cross-section at breakpoint or approximately midpoint of bone.

a,b,c Values in same column with different superscripts are different ($p < .05$).

Bone mineral content 1 cm below the nutrient foramen was greater ($P<.05$) in the high iron supplemented group (table 17). Differences were undetected by photometric densitometry. Morphometric measurements from radiographs were used to calculate the circular area index and differences ($P<.05$) were noted between the control, 500 mg/kg iron supplemented group and the high iron group.

Dietary iron supplementation did not appear to be detrimental to bone development at the levels fed in this study. Early reports established a negative relationship between high iron intake and bone mineral content. Cox et al. (1931) reported that when iron was fed to guinea pigs at levels greater than phosphorus the ash, calcium and phosphorus of bones was reduced. Brock and Diamond (1934) and Rehm and Winters (1940) also reported excess dietary iron lowered bone ash and produced evidence of rickets in rats.

O'Donovan et al. (1963) and Parugnai (1972) noted signs of phosphorus deficiency in growing pigs. Both studies reported lowered bone ash and serum phosphorus when iron was supplemented at 5000 mg/kg or greater. Lowered serum phosphorus in ruminants at much lower iron levels suggest they are more sensitive to iron-phosphorus antagonisms than non-ruminants (Standish et al., 1971).

Poultry appear to be more tolerant to high iron than chickens, turkeys, rats, guinea pigs, cattle and sheep and may metabolize iron more like pigs.

Treatment differences in BMC may be explained by non-uniformity of weight of the pouts assigned to treatments. The standard deviation for the final weights of the pouts were control, 231 $\pm$ 50, 500 mg/kg iron

supplemented group, 282 ± 110 and high iron group 281 ± 24 kg. Due to the great variability in weights of the control group and 500 mg/kg iron supplemented group each having one small pony, there may be expected to be differences in BMC.

Mechanical forces are thought to be responsible for stimulating bone growth and mineralization (Lanyon, 1982). Therefore, ponies of greatest and more uniform weight, that are of similar breeding and managed under similar conditions, may be expected to have higher BMC because of greater forces acting upon bones.

This suggestion may be supported by differences seen in breaking load and cortical area (table 17). Breaking load is the amount of force applied at bone failure, and is directly related to BMC (Grandhee et al., 1981).

Photon absorption estimates of BMC accurately reflected changes seen in BMC determined by sawing the metacarpals and their breaking load. Ultrasound, a technique developed to predict breaking strength, showed no differences in velocity measurements. Photometric densitometry failed to reflect differences in BMC by circular area indices calculated from caliper measurement did reflect differences in the high iron supplemented group (table 18).

In conclusion, 1000 mg/kg supplemental iron does not appear to affect growth or bone development of young ponies. However, interactions between the elements iron, copper, zinc and manganese need further study.

TABLE 18. EFFECT OF DIETARY IRON ON PHOTOMETRIC DENSITOMETRY ESTIMATES OF BONE MINERAL CONTENT AND CORTICAL AREA OF THIRD METACARPALS OF PONIES[1]

Supplemental Iron mg/kg	BMC g	Photometric Densitometry	Circular Area Index
0	5.68[b]	8.74[a]	4.35[b]
500	5.76[b]	9.05[a]	4.05[b]
1000	7.15[a]	9.69[a]	5.53[a]

[1]Measurements determined on 2 cm cross section 1 cm below the nutrient foramen.

[a,b,c]Values in same column with different superscripts are different ($p<.05$).

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

E. E. Ott, Chairman
Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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